

**Morphology Control for Particle Stabilized Droplets and Droplet
Templated Microcapsules**

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Abiola Shitta

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Morphology Control for Particle Stabilized Droplets and Droplet Templated Microcapsules

Approved by:

Dr. Sven H. Behrens
School of Chemical & Biomolecular
Engineering
Georgia Institute of Technology

Dr. Carson Meredith
School of Chemical & Biomolecular
Engineering
Georgia Institute of Technology

Dr. Victor Breedveld
School of Chemical & Biomolecular
Engineering
Georgia Institute of Technology

Dr. Yulin Deng
School of Chemical & Biomolecular
Engineering
Georgia Institute of Technology

Dr. Alberto Fernandez-Nieves
School of Physics
Georgia Institute of Technology

Date Approved: November 4th 2015

I would like to dedicate this thesis to my parents, Owolabi and Muyinot Shitta, who have always encouraged me to be nothing less than the best that I can be.

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SUMMARY

This thesis investigates new uses and benefits of colloidal particles adsorbing to the liquid-liquid interface of emulsion droplets. The first part of this thesis investigates morphology control of double emulsions of the water-in-oil-in-water (W/O/W) variety due to the use of interfacially adsorbing particles. W/O/W emulsions typically yield oil droplets containing a multitude of internal water droplets when scalable emulsification procedures are used. W/O/W emulsions containing a single internal aqueous core are preferred for some applications; but single core double emulsions usually require small-scale continuous processes that have low yields. As a result of the investigation into double emulsion morphology control, a readily scalable batch procedure for the production of single core droplets was developed that requires particulate emulsifiers of appropriate concentration and wettability as well as osmotically induced coalescence of the internal droplets. The second part of this thesis describes a different method of morphology control applied to pairs of immiscible droplets brought into contact in a liquid medium. It was found that colloidal particles adsorbing selectively to the different liquid interfaces can change the balance of interfacial tensions responsible for the wetting morphology of the two droplets. Therefore, particles can be used as “wetting modifiers” that allow for control of the equilibrium droplet configuration in a way that was previously considered achievable only with the help of surfactants. The third and final part of this thesis explores the use of particle stabilized emulsion droplets in a new strategy for microencapsulation. In collaboration with an industrial research sponsor, double walled microcapsules with polymer-particle composite shells were developed.

The composite shells were formed by interfacial polymerization at the particle covered liquid interfaces. The developed microcapsules were characterized with regard to their potential benefits for the storage and sustained release of aqueous cargoes.

CHAPTER 1

INTRODUCTION

1.1 The Encapsulation Starting Point

Encapsulation phenomena occur in a wide variety of places in nature. Egg shells, plant seeds, bacterial spores, and sea shells are some examples of naturally occurring protective capsule layers [1]. A little over forty years ago, some of the first industrial micro-encapsulation methods were developed by taking lessons from what nature has already done. These encapsulation methods are currently used to make food, pharmaceutical, cosmetic and agricultural products. The most notable use is in the pharmaceutical industry where capsules are used for prescription drugs, over the counter drugs as well as vitamins and minerals [1].

Encapsulation is used to serve many functions such as separation of incompatible ingredients and protection of reactive substances. The resulting capsule also serves as a means through which controlled, targeted or triggered delivery of the capsule contents can occur. The ability to deliver an active ingredient over an extended period of time has been shown to be an effective way to improve the efficacy of the active in comparison to a similar dose of the active without encapsulation in some applications [2]. The encapsulation of liquid droplets represents a particular versatile formulation strategy due to its applicability to all actives soluble or dispersible in a given liquid and avoids many of the challenges associated with specific interactions between the active and the capsule wall material. Moreover, emulsion droplets of any liquid are easily formed using well-known technologies and provide a convenient template for capsule formation and the immersion of many actives in an appropriate liquid promotes their bioavailability.

In the case where an aqueous cargo is to be protected or delivered in an aqueous environment, the droplet-in-a-droplet morphology of W/O/W (water-in-oil-in-water) double emulsions has proven particularly useful [3, 4]. Double emulsions have the benefit of having an additional phase that can separate the internal phase from the environment. This additional phase can be solidified, extracted, or exploited for its two interfaces which can be used for interfacial polymerization [5-7]. Our interest in encapsulation came about due to an industrially sponsored project where we were tasked with developing a method for encapsulating aqueous agrochemicals. The industrially sponsored project also gave us the opportunity to investigate other topics of interest to our group which became additional projects that will be discussed in this thesis.

1.2 Use of Pickering Particles

In order to stabilize the emulsion templates required to make double emulsion based capsules, surfactants or particles can be used. Surfactants are the more typical emulsion stabilizer and have long been studied. Particle stabilized emulsions which are also called “Pickering emulsions” were first discovered a little over one hundred years ago [8, 9], and have only attracted attention substantial scientific attention in the past two decades. Emulsions stabilized by particles have benefits over surfactant stabilized emulsions with the most important benefit being the greater energy of adsorption associated with the particles in comparison to the surfactant [10, 11]. This leads to a greater amount of long term stability due to their irreversible adsorption to the liquid interface.

Particles can be made from a variety of materials such as silica, clay minerals or calcium carbonate. All of these examples are environmentally benign, abundant and

cheap and hence desirable materials from which emulsions can be stabilized. Renewed interest in particle stabilization has occurred due to the new types of particles that can be prepared uniformly and with a wide variety of functionality on the particle surface. This functionality makes it possible for emulsions and emulsion templated capsules to have added responsiveness to external stimuli such as temperature, pH or ionic strength [6]. Throughout this thesis, silica particles will be used as the interfacial stabilizer. Each of the projects discussed in this thesis will demonstrate how the use of particles provides some benefit or novel method to achieve an end goal. The following sections will setup each of the main thesis chapters (Chapters 2-4) and highlight the significance of the projects undertaken as well as the challenges that were addressed.

1.3 Improving Bulk Double Emulsion Preparation

When emulsions are prepared via batch emulsification, there is very little control over the droplet size and morphology; which so far limits the commercial use of emulsion templated capsules [12]. Microfluidic devices have been used to produce double emulsions with a very high degree of control over the size distribution of the resulting droplets [13-15]. While a high degree of control over the droplet size distribution is highly appealing, some large scale industrial applications will not be viable with this method of droplet production since flows on the order of $\mu\text{L}/\text{min}$ per device are used. Due to the low throughput of microfluidic devices, an alternative method that has a degree of control of the size and morphology of emulsion droplets that can be used in large batches is highly attractive.

When double emulsions are produced in bulk, two separate emulsification steps are required. In the case of a W/O/W double emulsion, the first emulsification step

produces a W/O (water-in-oil) emulsion. The first emulsification step is simple. There are no limitations to the amount of shear applied and it is much easier to produce droplets with a narrow size distribution. The second emulsification step, however, is much more difficult. Only a limited amount of shear can be applied during the second emulsification step and it is much less likely for the droplets to be uniform in size. Figure 1.1 depicts the two emulsification steps required to make a double emulsion.

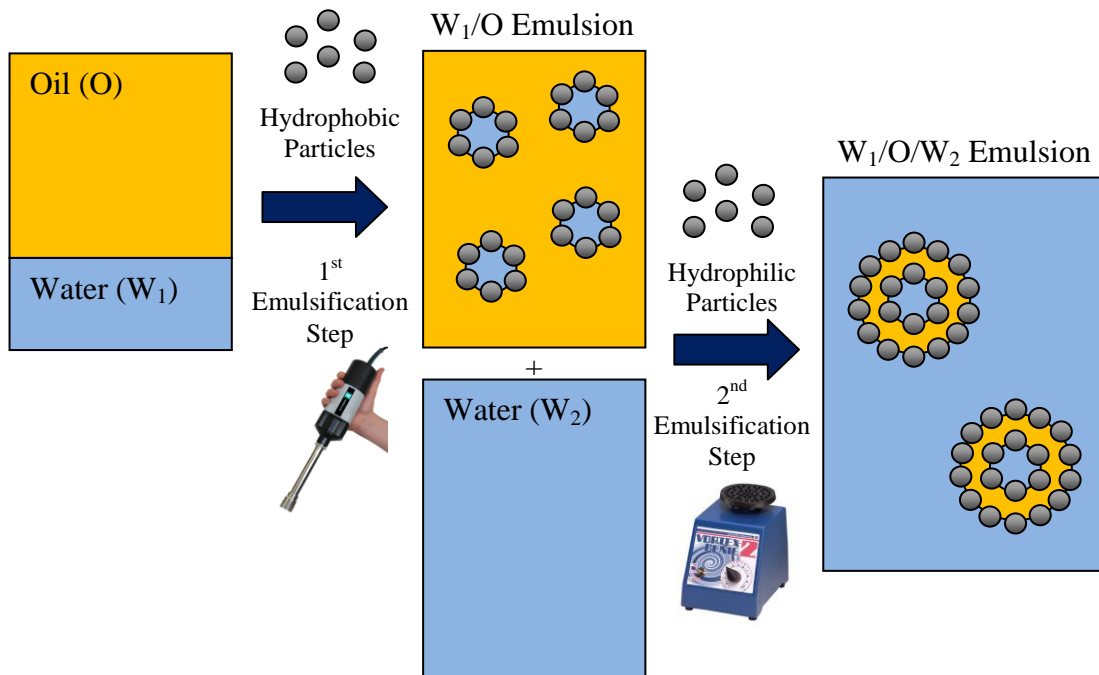


Figure 1.1: Depiction of the two emulsification steps required to make a double emulsion

The challenge of producing uniform emulsion droplets with conventional rotor-stator homogenizers, or a vortexer for that matter, is based on an inability to subject each individual droplet to a uniform shear field. When a uniform shear field is applied to emulsion droplets, it is easy to produce an emulsion that is monodisperse [16, 17]. Microfluidic devices and Couette mixers are examples of tools that produce uniform shear fields and can hence produce monodisperse emulsions. While microfluidic devices

and Couette mixers are very capable of producing monodisperse emulsions, their industrial implementation would require that new equipment be installed. The installation of new equipment is not necessarily problematic; but if alternative methods can be used that avoid the installation of new equipment, the alternative method is much more likely to be used.

Chapter 2 of this thesis discusses our contribution that is relevant to the production of double emulsions produced in bulk. While it is not possible to shear double emulsion droplets significantly enough using a conventional rotor-stator homogenizer, it is possible for steps to be taken afterwards to encourage uniformity in the number of internal droplets. The number of internal droplets in a double emulsion is one measure of double emulsion stability. If the double emulsion loses its contents to the external bulk phase, the number of internal droplets decreases. This is just one of the ways that destabilization can happen in a double emulsion. Figure 1.2 depicts some of the paths that a double emulsion droplet can take as it is destabilized due to either droplet coalescence or diffusional water transfer.

Since the desirable double emulsion morphology for encapsulation has a single, large internal aqueous core, it would make sense to encourage coalescence of the internal droplets. However, coalescence can lead to destabilization of an emulsion and ultimately separation of the phases if it proceeds too far. Chapter 2 of this thesis explains how we were able to facilitate coalescence of the inner droplets without destabilizing the double emulsion and ultimately produce double emulsion droplets that had a single core using particles as the emulsion stabilizer.

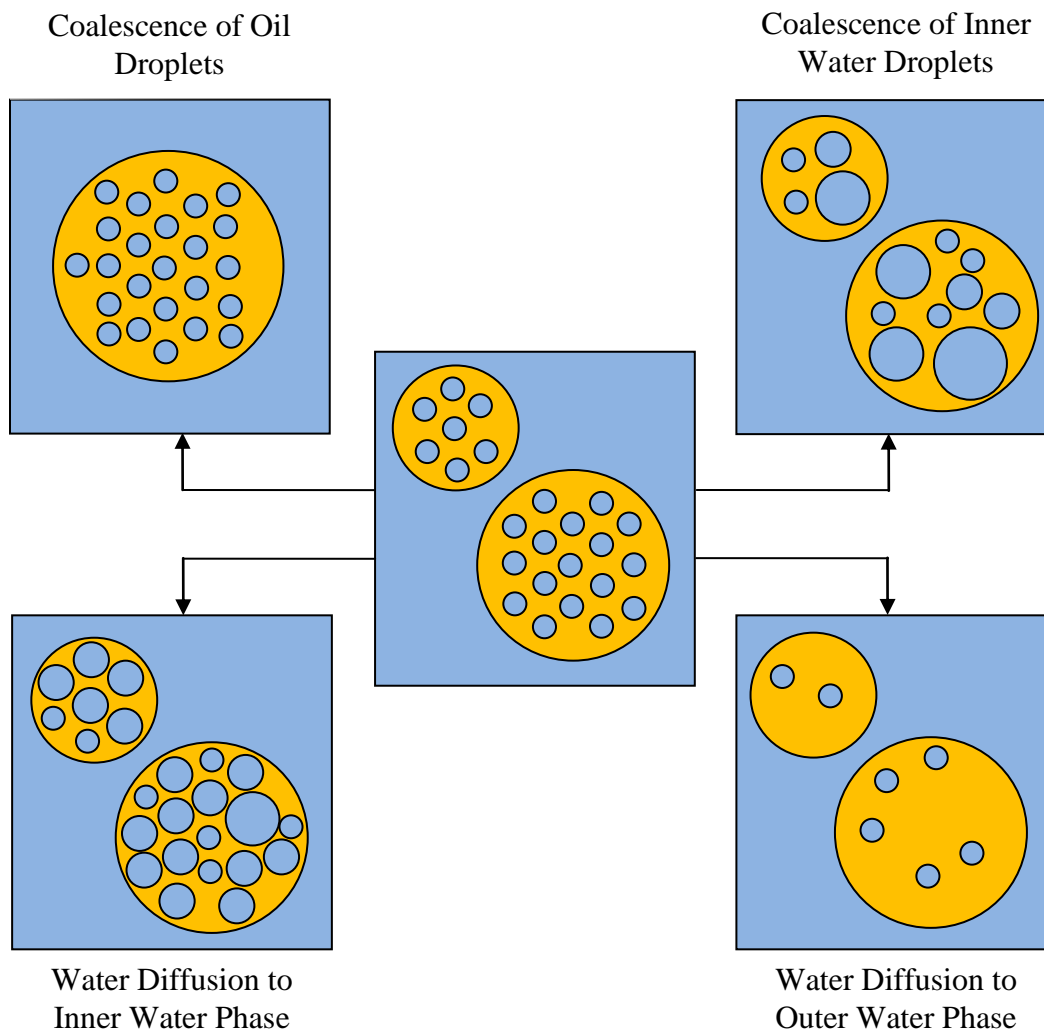


Figure 1.2: Pathways to destabilization for a double emulsion droplet

1.4 Beyond the Droplet-in-a-Droplet Morphology

The droplet-in-a-droplet morphology was used in this thesis as a capsule template for encapsulation applications. However, there are alternative uses for templated droplets. There a variety of colloidal particles that differ in their morphology that can be produced from droplets [18]. Figure 1.3 depicts some of these particles.

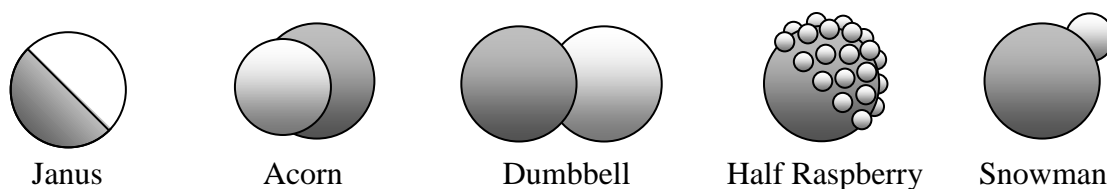


Figure 1.3: Colloidal particles that can be produced from templated droplets

The most common of the colloidal particles, shown in Figure 1.3, is the Janus particle; a particle that has two hemispheres that are functionalized differently. A Janus particle has a greater adsorption energy than a homogeneous Pickering particle and it has been theoretically proven, although not practically demonstrated, that Janus particles can produce thermodynamically stable emulsions [19, 20]. We never intended to make Janus particles or other colloidal particles. In the pursuit of methods to make uniform double emulsion droplets, a novel method of changing the morphology of contacted immiscible liquid droplets was discovered.

Changing the morphology of immiscible liquid droplets is not a new phenomenon [21, 22]. The fundamental principles that determine how contacted liquid droplets wet one another are also relevant to the production of hollow latex particles used for pigments [23, 24]. What is novel about our discovery is the way that the contacted droplet morphology is being changed. We discovered that the adsorption of Pickering particles to a specific droplet interface was sufficient to change the equilibrium droplet morphology. All of the literature published on changing droplet morphology, until very recently by our group [25], has used surfactants to change the equilibrium droplet structure. This is a non-trivial discovery because, based on what we know about Pickering particles, particles are not expected to significantly impact interfacial tension; a parameter that determines the resulting equilibrium morphology.

The only clear preceding evidence of particles changing morphology used particles adsorbed to bulk interfaces to demonstrate what was termed “particle assisted wetting.” [26-28]. In the “particle assisted wetting” experiments, it was very clear that the particles provided an energetic benefit at the interfaces they adsorbed to. The energetic benefit that the particles provided was sufficient to spread an oil at an air-water interface that would otherwise form lenses in the absence of particles. In the experiments where droplets are contacted in comparison to bulk interfaces, no experiments (until recently by our group) have been published that use Pickering particles to change morphology. In the work done on “particle assisted wetting”, the water-oil-air system that was used only required a slight energetic reduction to change the morphology. In addition, a Langmuir trough was used to ensure that the particles were packed as densely as possible to maximize the impact of particle adsorption. If other three phase systems are used that, for example, have all liquid phases, it is not as likely that a clear change in morphology will occur. When three liquids are used, it is much more difficult to make the particles adsorb to the interfaces necessary to cause a morphology change. Since droplets are being contacted instead of bulk interfaces, it is also more difficult to ensure a dense packing of particles since a Langmuir trough cannot be used. We thought that it was important to explore what Pickering particles are capable of when the more challenging three phase liquid system is used as the platform for morphology change.

Chapter 3 of this thesis describes our contribution to the understanding of Pickering particles and their ability to change droplet morphology. Using three phase systems of mutually immiscible liquids, in comparison to two liquid phases and one gas phase, we have demonstrated that not only can particles change the morphology of the

interfaces they make contact with; particles can also significantly change the interfacial tensions of the interfaces they adsorb to. The latter statement is not a widely accepted belief. Hopefully the evidence from this work as well as other published work by our group will start to serve as evidence that Pickering particles can significantly change interfacial tension.

1.5 The Industrially Sponsored Project

As was mentioned earlier, the industrially sponsored project focused on encapsulation of aqueous herbicides was the driver for all of the other projects that were pursued in this thesis. The encapsulation of agrochemicals like herbicides, fungicides and pesticides has been investigated for some time [12]. Some agrochemicals contain compounds that are toxic and, for this reason, it is imperative to keep these compounds encapsulated. Encapsulation, when applied to agrochemicals, also serves the purpose of minimizing unwanted damage to non-target crops that would typically occur if the agrochemical was sprayed. The most common encapsulation scheme involves O/W (oil-in-water) emulsion templates. The use of O/W templates is due to the prevalence of commercial O/W emulsion food products like milk, cream, salad dressing and many others that make use of encapsulated ingredients [29]. Hydrophobic agrochemicals can easily be encapsulated in an O/W emulsion template. However, encapsulation of hydrophilic actives of any type is much less common [30]. The primary concern of aqueous based encapsulation is solubility. The encapsulated aqueous component is very likely to be exposed to an aqueous environment in which the active is also soluble. The aqueous active, due to its exposure to the aqueous environment, will easily be released from the capsule and washed away from its target. For obvious reasons, solubility

concerns are less relevant to the encapsulation of hydrophobic actives that are intended for use in aqueous environments.

In order for an aqueous active to be effective, the employed method of encapsulation needs to sufficiently inhibit diffusion of the active ingredient to the aqueous environment. In addition, the active ingredient also needs to be released with a release profile that will have an impact on the target. Depending on the properties of the target, the release profile may need to very suddenly deliver the active while in other cases delayed, gradual and sustained delivery of the active is required. One method of making capsules, that is versatile and that can provide sufficient diffusional resistance to the release of the active, involves the use of double emulsions. For our purposes, the double emulsion would have to be of the W/O/W variety. W/O/W double emulsions have been used to encapsulate aqueous based ingredients with great success [3, 30]. The clear benefit of using a W/O/W emulsion is that the oil layer provides a barrier to diffusion that can, in addition, be solidified, extracted or its interfaces used for interfacial polymerization.

In order to demonstrate the viability of an aqueous active encapsulation method, the aqueous herbicide used for the majority of our experiments was CLARITY®. CLARITY® was used because it was important for us to demonstrate that a method of encapsulation could be developed that was compatible with an aqueous herbicide formulation. Chapter 4 of this thesis describes our contribution to the encapsulation of aqueous actives. We developed a novel method of aqueous encapsulation that uses a W/O/W double emulsion template. The double emulsion template was stabilized by particles and the liquid interfaces were interfacially polymerized. Considerations about

the active ingredient, adjuvants and the monomers used are discussed as well as their impacts on the resulting capsule performance.

CHAPTER 2

MORPHOLOGY CONTROL VIA OSMOTIC SWELLING

2.1 Background

Producing double emulsions in industrially relevant quantities and ensuring that the droplets are uniform is a challenge. In order for double emulsions to be produced on a large scale, affordable and effective methods have to be used. For this reason, homogenizers are very common in industry. Homogenizers, however, have a difficult time producing double emulsion droplets that are sufficiently monodisperse in size and morphology. It is particularly difficult to have any control over the morphology of double emulsion droplets during homogenization. In the case of W/O/W encapsulation applications, the desirable double emulsion morphology has a single aqueous core and a uniform oil layer surrounding the core. This morphology, the droplet-in-a-droplet morphology, is ideal because the release of the active from the double emulsion is predictable and it has been demonstrated that adjusting the thickness of the surrounding oil layer changes the capsule release profile [15]. In some applications a broad size distribution may be permissible. But when double emulsions are being used in applications that require a narrow size distribution, like slow release capsules, polydispersity can be problematic. The polydispersity in slow release capsules would result in capsules with varying wall thickness and hence an unpredictable release of the encapsulated ingredients.

2.2 Objective

The initial idea, for the industrially sponsored project on aqueous active encapsulation, was to make double emulsion droplet templates that have a uniform oil layer surrounding the internal aqueous phase. These templates would serve as the foundation for the formation of slow release capsules that contained the aqueous active ingredient. This chapter describes how the pursuit of double emulsion droplets that contain a single aqueous core led to a novel methodology by which particle stabilized double emulsions prepared in bulk can be made to possess a single aqueous core.

2.3 General Approach and Rationale

When we started this project, there was no preceding literature on double emulsions that could be prepared in bulk that also possessed a single internal aqueous droplet. Our initial approach to producing uniform double emulsion droplets pulled from an understanding of Pickering particles and their ability to assist in oil spreading [26, 27, 31]. Oil spreading did not ultimately play a role in the developed method of morphology control discussed in this chapter. Unfortunately, the use of particle assisted spreading to change the double emulsion droplet structure does not lend itself to use in the bulk production of double emulsions. The following chapter, Chapter 3, deals with a project that resulted from attempting to use Pickering particles to induce morphology changes in O/O/W (oil-in-oil-in-water) systems that took advantage of particle assisted oil spreading. The successful approach of bulk morphology control was based on the idea that double emulsions with a single internal aqueous core could be formed after homogenization had already taken place. Attempting to force the droplet morphology to change during homogenization is ineffective due to the fact that batch homogenizers are limited in their

ability to produce a uniform shear field. The lack of a uniform shear field will undoubtedly lead to double emulsions that have a variety of droplet sizes and morphologies. Since the droplet size of double emulsions cannot be controlled during homogenization, as was previously stated, the number of internal droplets is a realistic property of the double emulsion that can be controlled after homogenization is complete. Changes in the number of internal droplets of double emulsion droplets have been investigated due to their direct relation to the emulsion stability of emulsion based commercial products [3]. A double emulsion that changes in morphology either by reduction of the number of internal aqueous phase droplets or coalescence of the internal droplets is deemed unstable and will not maintain the intended morphology. Double emulsions have unique stability problems due to the fact that two different emulsifiers are required for stability. In the case of a W/O/W double emulsion, a hydrophobic emulsifier is required to stabilize the inner W/O interface and a hydrophilic emulsifier to stabilize the outer O/W interface. When surfactants are used as the emulsion stabilizer, this creates an innate instability problem. Surfactants reversibly adsorb to the interfaces they may contact with and are hence in a constant dynamic state of adsorption and desorption [10, 11]. This creates opportunities for the hydrophobic surfactant, intended to stabilize the inner W/O interface, to demulsify the outer O/W interface. Similarly, the hydrophilic surfactant, intended to stabilize the outer O/W interface, will demulsify the inner W/O interface. In addition, it is possible for reverse micelles of the hydrophobic surfactant to form. These reverse micelles can stabilize water droplets in the oil phase and transfer the aqueous contents of the double emulsion to the external aqueous bulk phase. While it has been shown that lowering the concentration of the hydrophobic emulsifier can reduce the

amount of reverse micelles, the inner droplets are innately less stable due to the fact that less surfactant is used [32]. Destabilization of the double emulsion via reverse micelles of the hydrophobic emulsifier is difficult to avoid. Osmotic gradients between the bulk and the internal aqueous phase are an additional concern for the stability of double emulsions that can further drive surfactant transfer to undesirable interfaces.

Osmotic gradients are very important in double emulsion systems. High osmotic gradients can cause catastrophic morphology changes which can lead to rupturing or osmotic transfer of the internal aqueous phase to the bulk [33]. Oil phases with a higher residual aqueous solubility are much more susceptible to destabilization in this manner. Because of this issue, osmotic gradients are typically minimized in order to improve the stability of the double emulsion formulations. Florence and Whitehill did pioneering work investigating the breakdown of double emulsion droplets [34]. This work, as well as others [32], has shown that the two main processes that destabilize surfactant stabilized W/O/W double emulsions are the aforementioned destabilization mechanisms of solubilization of internal aqueous phase via reverse micelles of the hydrophobic emulsifier and transfer of water either in or out of droplets via osmotic gradients [35]. Double emulsions stabilized by surfactants can be destabilized by both of the destabilization mechanisms. Our work shows that double emulsions stabilized by particles, also known as Pickering particles, are not as easily destabilized and provide a means by which a novel degree of morphology control can be observed.

Pickering particles were discovered over 100 years ago and have since been used as an alternative emulsion stabilizer to surfactants [8, 9]. Recent interest in these particles has grown due to advances in the production of monodisperse particles that have a wide

variety of functionality on the particle surface [6]. It is well known that Pickering particles adsorb to interfaces with high adsorption energy and that they can arrange themselves on an interface to minimize interfacial area between phases [36]. Due to their high adsorption energies, the particles adsorb irreversibly to the interfaces they have preferential wetting for. The fact that Pickering particles adsorb irreversibly to these interfaces means that they cannot solubilize the internal phase like surfactants. As a result, destabilization of the emulsion via solubilization of the internal phase is not an issue and the osmotic gradient alone drives the morphological changes.

While morphological changes in double emulsions are typically viewed as a problem, they present an opportunity, and hence our contribution, for favorable morphology changes to occur in the case of particle stabilized double emulsions. Osmotic gradients cannot be impeded solely by using particles. But using a lower concentration of particles makes it possible for the osmotic gradient to serve as a means to accelerate internal droplet coalescence and make desirable morphological changes happen faster. Using the combination of Pickering particles and limited coalescence accelerated by osmotic swelling of the internal aqueous droplets, we have demonstrated control over bulk double emulsion droplet morphology that can improve the viability of double emulsions produced in bulk.

2.4 Experimental Section

2.4.1 Phase Preparation

CLARITY® (BASF) is used as the internal aqueous phase of the double emulsion. CLARITY® has some desirable properties that make it a reasonable internal aqueous phase candidate. CLARITY® is green in color which we believed would aid in

optical observation and contains a salt, dicamba-DGA, which would aid in driving osmotic swelling. CLARITY® is by no means the only aqueous phase that can be used for osmotic swelling. Any aqueous phase that is stabilized with Pickering particles that has sufficient wettability for the inner W/O interface should work. The dicamba-DGA salt present in CLARITY® is not sufficient to drive osmotic swelling at a reasonable rate. For this reason, 0.5 M NaCl (s) is added to CLARITY® to increase the rate of osmotic swelling.

Trimethylolpropane trimethacrylate or TMPTMA (SIGMA) is used as a photo-polymerizable oil phase that makes it easy to maintain the droplet morphology and observe the resulting droplet structure with microscopy. The oil is first prepared by adding 1-2 wt% of aluminum oxide (SIGMA) and then stirring continuously for 24 hours. The mixture is then left to sit for another 12 hours to allow the aluminum oxide to sediment. TMPTMA is then decanted from the aluminum oxide and 0.1-0.5 wt% of hydrophobically modified silica particles, Aerosil® R805 (EVONIK), are added. Finally 0.1 wt% of benzoin isobutyl ether (TCI America) is added as a photo initiator. The external aqueous phase is prepared by simply adding 2 wt% of hydrophilic silica particles, Aerosil® 300 (EVONIK), to DI water.

2.4.2 Double Emulsion Preparation, Swelling and Deswelling

0.75 mL of CLARITY® with added NaCl is added to 1.5 mL of the prepared TMPTMA oil phase and homogenized using a rotor-stator homogenizer (IKA Ultra Turrax T10) at 30,000 rpm for 30 seconds. This W/O emulsion is then poured into 3-5 mL of the external water phase. The mixture is then re-emulsified using a vortexer (Vortex Genie 2) for 30 seconds which completes preparation of the W/O/W double

emulsion. The double emulsion is then placed in a covered Petri dish and left to sit for 1 hour. Once an hour has passed, the double emulsion is osmotically swollen.

In order for deswelling to be successful, the concentration of salt in the external aqueous phase must exceed the salt concentration in the internal aqueous phase. In addition, the salt concentration in the external aqueous phase has to be increased gradually to avoid osmotic shock. We achieved osmotic deswelling by gradually adding a 1-2 M NaCl solution, with a syringe pump, to the aforementioned double emulsion. The double emulsion is left to sit for 3 hours or until the internal aqueous core has reached the desired size.

2.4.3 Oil Phase Solidification and Observation

Once the double emulsion has undergone swelling and/or deswelling, the oil phase can be solidified. The double emulsion is placed in a covered Petri dish and exposed to UV light (365 nm) for a period of 10 minutes. Once this is done, the solidified double emulsion can be redispersed in water and observed with bright-field microscopic methods.

2.5 Results and Discussion

The procedure of making double emulsions by two step batch emulsification requires special caution. During the first homogenization step, there is no limit on the amount of shearing that can be used. During the second emulsification step, high amounts of shearing are not advised since too much shearing will rupture the double emulsion droplets and produce a single emulsion. For these reasons, the typical starting double emulsion has multiple internal aqueous droplets as can be seen in Figure 2.1.

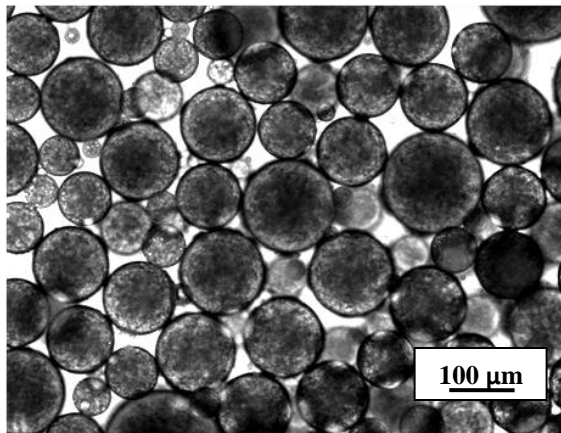


Figure 2.1: W/O/W double emulsion droplets with multiple aqueous cores (Scale bar is 100 μm)

In order to prepare double emulsion droplets that have a single internal aqueous core, a morphology that is desirable for encapsulation application, we pursued a method of controlled coalescence of the internal aqueous droplets.

2.5.1 Particle Concentration Impact

Coalescence of the internal aqueous droplet can be encouraged by reducing the concentration of hydrophobic particles in the oil phase. At a certain lower particle concentration, the emulsion shifts into what is called the “emulsifier poor” regime of “limited coalescence” [37]. When the particle concentration is lowered into the “emulsifier poor” regime, coalescence proceeds until the largest interfacial area that can be protected against coalescence is stabilized. In the “emulsifier poor” regime, the particle concentration ultimately dictates the mean drop size during emulsification [11, 38]. This is in contrast to the case where high concentrations of particles are used and the emulsion is in the “emulsifier rich” regime. In the “emulsifier rich” regime, the maximum droplet coverage is reached before all of the dispersed particles have adsorbed and hence coalescence is limited by the rate of particle adsorption rather than the total number of

particles. Figure 2.2 depicts droplet coalescence and the different particle adsorption events that can occur. For the system considered in the present study and the chosen intensity of homogenization, particle concentrations on the order of 0.5 weight percent or less were required to produce a double emulsion that undergoes a significant amount of coalescence. Figure 2.3 shows a double emulsion prepared with a reduced amount of hydrophobic particles in the oil phase in comparison to one prepared with a greater amount of hydrophobic particles.

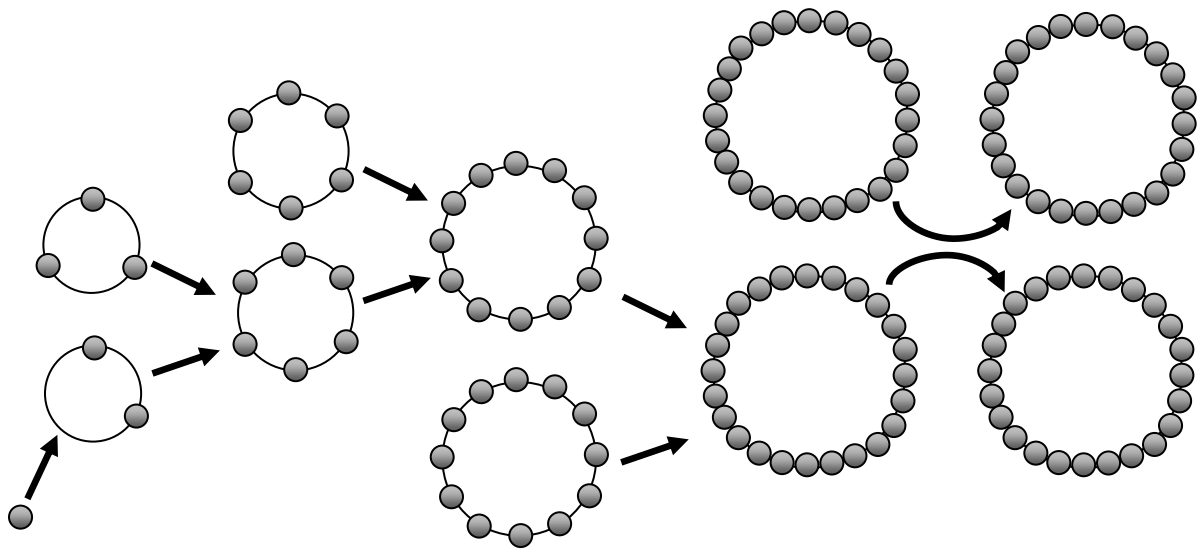


Figure 2.2: Depiction of “limited” droplet coalescence proceeding until the droplets possess the maximum allowable particle coverage.

Figure 2.3 shows that reducing the particle concentration from 2 wt% to 0.01 wt% reduces the number of aqueous core droplets found in a typical oil droplet, which indicates that coalescence has proceeded further at the lower particle concentration (“emulsifier poor”) regime. Not all of the internal aqueous droplets have coalesced at the lower particle concentration. This is likely due to the fact that the image was taken immediately after the double emulsion was made and coalescence did not have the opportunity to proceed further.

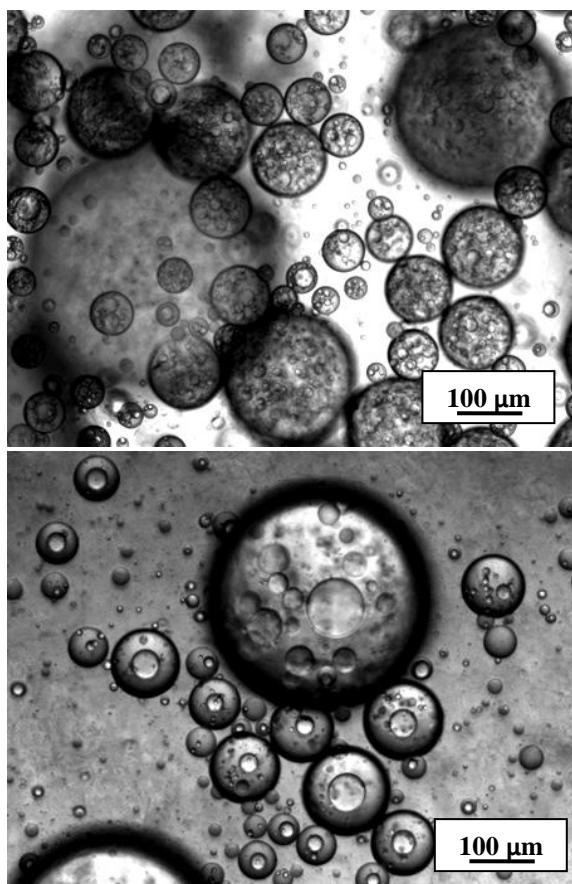


Figure 2.3: W/O/W double emulsion droplets with a particle concentration of 2 wt% (top) and 0.01 wt% (right) (Scale bar is 100 μm)

Lowering the particle concentration to less than 0.005 wt% destabilizes the internal aqueous phase during the second emulsification step, which releases the internal contents to the external aqueous phase during homogenization. Therefore an additional step is required to drive coalescence further yet still maintain a sufficient level of emulsion stability.

2.5.2 Osmotically Induced Coalescence and Deswelling

The use of an osmotic gradient, created by adding salt to the internal aqueous phase, makes it possible for water to further swell the internal aqueous droplets. This encourages more coalescence by increasing the interfacial area of the internal aqueous

droplets, reducing their particle coverage and ultimately creating a new driving force for coalescence. Osmotic swelling alone can drive some coalescence even if the particle concentration is high. But this is likely because droplet coalescence, which is accelerated by osmotic swelling, competes with the adsorption of excess particles as a means to reach the maximum allowable surface coverage. Figure 2.4 shows a double emulsion with a higher particle concentration after osmotic swelling has taken place. The internal aqueous cores increase in size but there are still some internal droplets that have not coalesced due to the high particle concentration.

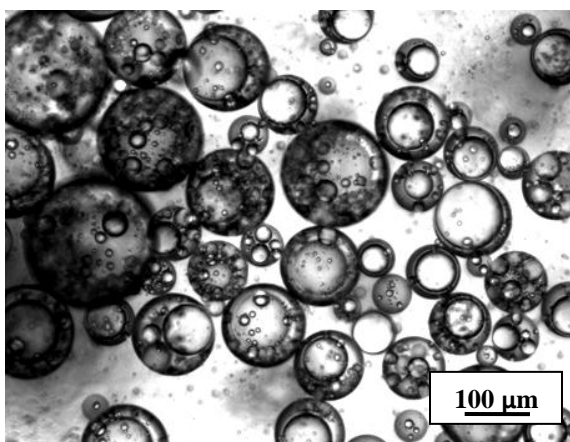


Figure 2.4: Double emulsion after osmotic swelling with a particle concentration of 1 wt% (Scale bar is 100 μm)

The most significant changes in morphology are observed when the double emulsion is prepared with a low concentration of particles stabilizing the inner W/O interface and osmotically swollen. Double emulsions are not typically prepared with low emulsifier concentrations in the “emulsifier poor” regime due to the expected destabilization of the interfaces of the double emulsion. Because of this, drastic changes in double emulsion morphology have not previously been observed when double emulsions have been osmotically swollen [33]. Figure 2.5 shows double emulsions and

capsules prepared with a low particle concentration after osmotic swelling has taken place. It is apparent that the majority of the double emulsion droplets contain a single aqueous core when they previously contained many aqueous cores.

The single internal aqueous core can subsequently be reduced in size using a reversed osmotic gradient. This is achieved by adding salt to the external aqueous phase which reverses the osmotic gradient and drives water out of the internal aqueous phase.

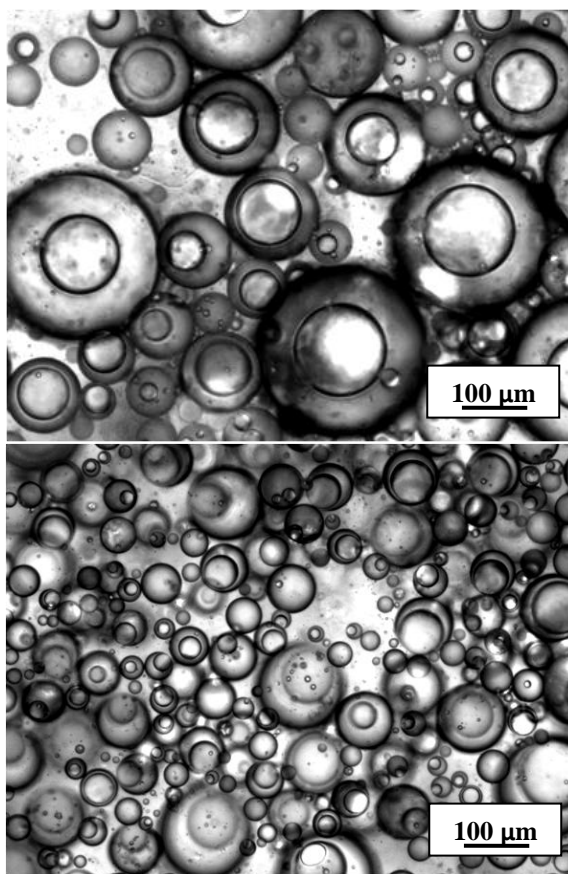


Figure 2.5: W/O/W emulsion droplets that have been osmotically swollen with a 0.1 wt% particle concentration (top) and capsules that have been prepared subsequently via photopolymerization (bottom) (Scale bar is 100 μm)

The particles are desorbed by force as the inner W/O interfacial area reduces irrespective of the high adsorption energy that the particles possess and has been previously observed [39]. When the osmotic gradient is reversed after the double emulsion droplets have been

osmotically swollen, the size of the internal droplets can be reduced significantly as can be seen in Figure 2.6. The overall process of droplet morphology change is depicted is summarized and depicted in Figure 2.7.

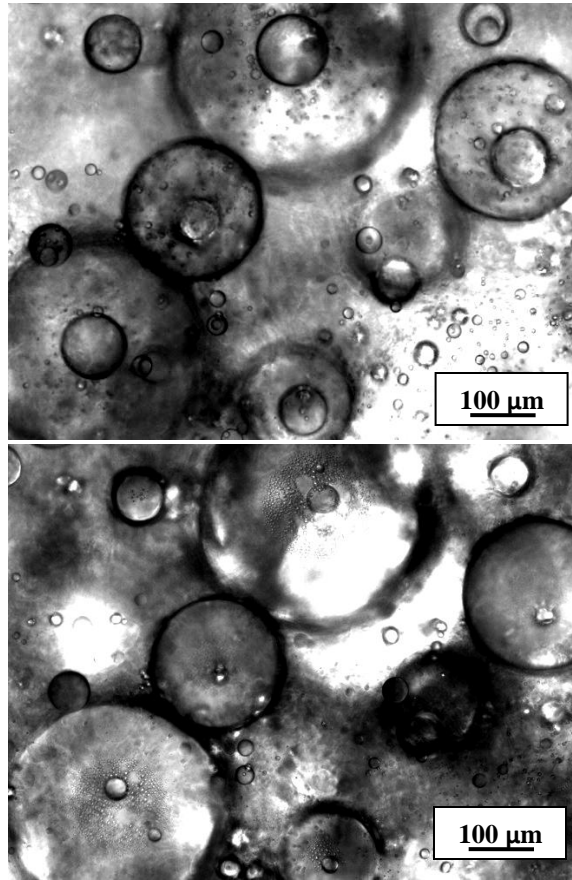


Figure 2.6: W/O/W emulsion droplets before (top) and after deswelling (bottom) (Scale bar is 100 μm)

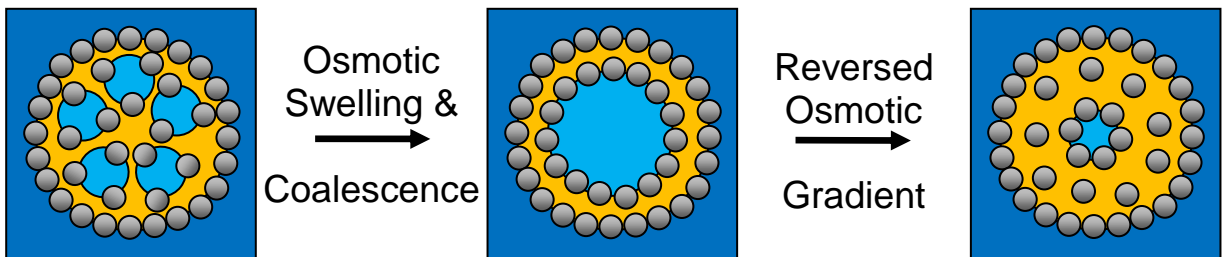


Figure 2.7: Depiction of changes in W/O/W double emulsion droplet morphology via osmotic swelling and deswelling

The initial double emulsion, containing multiple internal aqueous cores, is osmotically swollen due to the addition of salt to the internal aqueous core. This leads to the formation of a double emulsion with a single aqueous core. Reversing the osmotic gradient, by adding salt to the external aqueous phase, drives water out from the internal aqueous core and makes it possible to adjust the thickness of the surrounding oil layer. A patent (“Water/oil/water emulsions including oil droplets containing a single aqueous core”, Patent application No. PCT/US2014/072654, Publication No. WO/2015/103190, Publication date: July 7, 2015, International filing date: December 30, 2014) was filed for the developed method of bulk double emulsion morphology control due to its industrial relevance and significance.

2.6 Conclusions

In summary, particle-stabilized W/O/W double emulsions composed of oil droplets with a single aqueous core can be fabricated by standard batch emulsification followed by osmotic swelling. Although the achievable level of droplet morphology control cannot rival the control offered by microfluidic methods, it can present an attractive upgrade to classical, high volume emulsification technologies. The key mechanism is controlled coalescence of the internal aqueous droplets due to the low concentration of particles stabilizing the inner W/O interface. The demonstrated changes in double emulsion morphology require the combination of irreversible adsorption of the emulsion stabilizer and osmotic swelling of the inner phase to increase droplet surface area and trigger coalescence of the inner droplets. Coalescence is limited by the amount of stabilizer added to the oil phase. The combination of these factors makes it possible to prepare W/O/W double emulsion droplets with typically a single aqueous core and a

higher degree of uniformity than is commonly achieved by bulk emulsification with conventional homogenization equipment. The next chapter describes a project that resulted from attempting to use particle assisted oil spreading to make uniform double emulsions that possess a single aqueous core.

CHAPTER 3

PARTICLE STABILIZED EQUILIBRIUM DROPLET STRUCTURES IN THREE PHASE LIQUID SYSTEMS

3.1 Background

Three phase systems have applications in the fields of oil extraction, foams, coatings, separations and encapsulation [14, 40]. In the field of encapsulation, a three phase liquid system of interest is the double emulsion. Double emulsions are commonly used as templates for capsules that can be prepared batch-wise, using common homogenization techniques, or with microfluidic devices on a drop-by-drop basis [13, 17]. Regardless of the method of preparation, the double emulsion droplet is only kinetically stable for the duration of time necessary to make the capsule. While this serves the intended purpose of making a capsule, double emulsion droplets that are thermodynamically stable would create opportunities for the development of novel encapsulation formulations, emulsion micro-reactors and the synthesis of micro-polymeric colloids like Janus particles [18, 41]. Double emulsions are highly unlikely to form a thermodynamically stable microemulsion when they are prepared in bulk with conventional homogenization equipment. However, when double emulsions are prepared with microfluidic devices or when immiscible liquid droplets are brought into direct contact, thermodynamically stable double emulsion droplets can be prepared [14, 21, 22]. When two immiscible liquid droplets are brought into contact in the presence of a third immiscible liquid phase, one of three things will occur. Figure 3.1 shows the three possible droplet morphologies that result from the direct contact of two immiscible liquid droplets in the presence of a third mutually immiscible liquid phase.

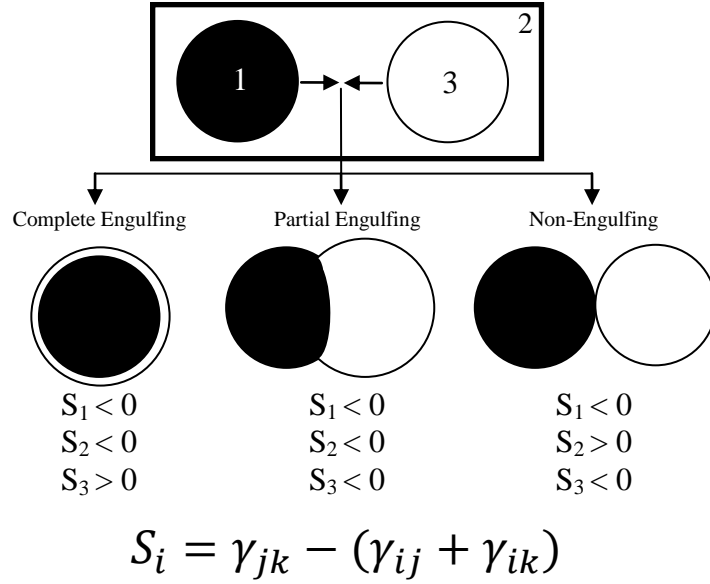


Figure 3.1: Illustration of the spreading coefficient conditions that result in various degrees of engulfing

The complete engulfing morphology, shown in Figure 3.1 on the left, is a desirable morphology for encapsulation due to the uniform layer surrounding the internal phase. Not all three phase liquid systems possess a complete engulfing morphology, and hence it is desirable to have a method by which the morphology can be changed. The only known approach to changing the morphology of the equilibrium droplet structure involves changing the interfacial tensions, expressed as γ in Figure 3.1.

In all previous work done with three phase equilibrium structures, surfactants were used to lower the interfacial tensions. Reducing the interfacial tension directly, via the use of surfactants, changes the spreading coefficients which can ultimately shift the three phase droplet morphology to a new equilibrium state. The phenomenon of shifting the morphology of a three phase droplet has not, until very recently by our group, been investigated with Pickering particles [25]. Pickering particles are alternative emulsion stabilizers that have gained increased recognition recently due to new developments in

particle synthesis and surface functionalization [6]. Pickering particles are not believed to significantly change the interfacial tensions of the interfaces where they adsorb. Yet experiments have shown that the presence of particles at an air-water interface can lead to the spreading of oil that would otherwise, in the absence of particles, form lenses at this interface [26-28]. More recently, it has also been demonstrated that oil can be spread around air bubbles due to the addition of adsorbing particles [25]. The fact that particles can cause visibly apparent changes to interfacial wetting behavior means that there is a clear energetic benefit, or a free energy reduction, to adsorbing particles in an interface. Silica particles are believed to only minimally change the interfacial tension [42]. Yet our work has shown that surface modifying silica particles makes it possible for the impact of particle adsorption on interfacial tension to be increased by an order of magnitude. Using surface modified silica particles, which typically have minimal impact on interfacial tension, we have demonstrated the first changes to three phase liquid droplet structures due to the adsorption of particles to the liquid interfaces.

3.2 Objective

In an attempt to make morphological changes to double emulsions prepared in bulk, the opportunity arose to investigate the possibility of forming a thermodynamically stable double emulsion droplet stabilized by particles. While a particle-stabilized double emulsion droplet in equilibrium was not produced, we were able to demonstrate that the addition of particles to a three phase droplet structure can shift the droplet to a new equilibrium state. This chapter explains how it is possible for the addition of Pickering particles to a three liquid phase droplet structure in equilibrium to create new equilibrium states. The phenomenon of producing new equilibrium states in three phase liquid

systems with particles has not been observed previously and this adds new fundamental understanding to what we know about Pickering particles.

3.3 General Approach and Rationale

The idea for this aspect of the thesis came from work done by Goedel et al. on particle assisted spreading [26-28]. In Goedel's work, the spreading of an oil, TMPTMA (trimethylolpropane trimethacrylate), at the air-water interface was facilitated by the preferential adsorption of Pickering particles. Particle assisted spreading is based on the theory that particle adsorption reduces the interfacial free energy of the interface the particles adsorb to. The reduction in free energy per unit area due to particle adsorption depends on the interfacial tension (γ) of the adsorbing interface, the particle contact angle (θ) and the particle packing density (ϕ) as shown in Equation 3.1.

$$\Delta G = \gamma(1 - \cos\theta)^2\phi \quad \text{Equation 3.1}$$

If the free energy reduction due to particle adsorption is significant relative to the spreading coefficient of the phase that it is desirable to spread, changes in the spreading behavior of that phase will occur [31]. Figure 3.2 shows a depiction of how the spreading behavior of TMPTMA at the air-water interface changes depending on the particle contact angle. The instances where spreading occurs are when the free energy reduction due to particle adsorption exceeds the spreading coefficient of TMPTMA.

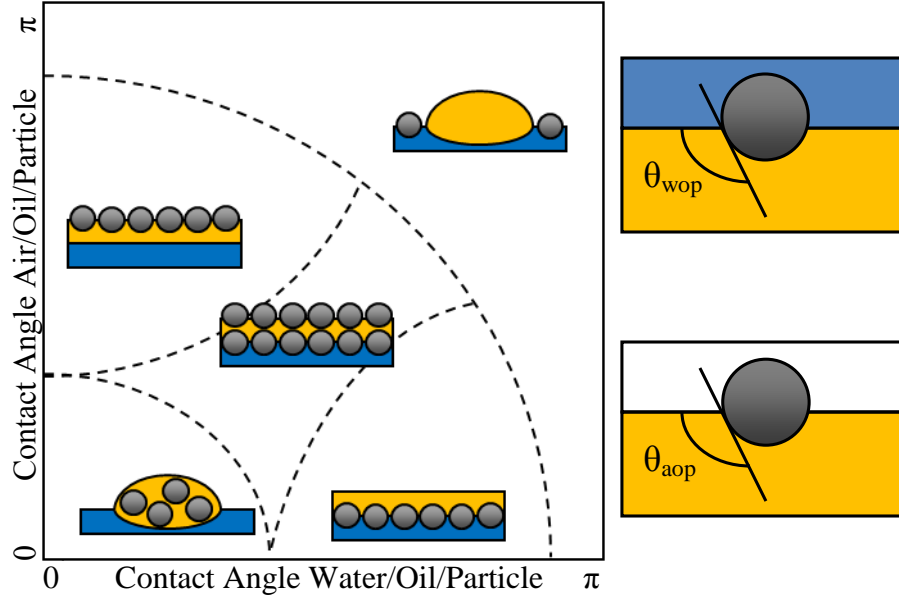


Figure 3.2: Illustration of a phase diagram for a macroscopic air-oil-water system and the possible morphologies that can result.

While Goedel's work involved macroscopic interfaces, we believed that particle assisted spreading could also be extended to small immiscible liquid droplets. We first had to prepare particles that would adsorb and that had favorable contact angles for spreading. In order to prepare particles that had the ability to adsorb and had an appropriate contact angle to reduce the interfacial free energy, the particles had to be surface modified. In our case as well as Goedel's, silica particles were used to create shifts in the equilibrium morphology. Silica particles are hydrophilic in their native state. Due to the hydrophilicity that silica particles possess, they may not adsorb to some oil-water interfaces. In order to improve the amount of adsorption of the silica particles, the silica particle were silanized. Silanization is a common method that is used to increase the hydrophobicity of silica surfaces [43, 44]. During silanization, silane compounds are covalently bonded to the original silanol sites which ultimately make the silica surface more hydrophobic. A variety of silane compounds can be used and each silane can impart

varying degrees of hydrophobicity. The surface modification can also make the particles preferentially adsorb to certain interfaces which depends on the liquid phases that are used.

In addition to preparing particles, a methodology by which three mutually immiscible liquid phase systems of differing morphologies could be generated was necessary. We developed a method capable of producing three phase systems with the aid of Krytox®. Krytox® is a perfluoropolyether that is immiscible in both water and most non-fluorinated organic compounds. All of the three phase systems investigated had Krytox® and water as two of the immiscible phases. The third phase could then be changed to produce a variety of three phase systems. For example, the third phase could be changed to dodecane, toluene or isobutyl acetate; each of which produces a different three phase equilibrium droplet structure.

With a means to generate three phase systems, a methodology also had to be devised to make particles preferentially adsorb to the appropriate interfaces. Our approach involved using surface modifying silanes that were either fluorinated or non-fluorinated. This approach is similar to a recent publication that used fluorinated and non-fluorinated surfactants to preferentially adsorb the surfactants to the different interfaces [45]. Particles that were modified with the fluorinated silane had preferential adsorption to the Krytox®-water interface. The particles that were modified with the non-fluorinated silane preferentially adsorbed to the non-fluorinated organic phase-water interface. If necessary, the particles could be modified with both of the surface modifiers to preferentially adsorb the particles to the Krytox®-non-fluorinated organic phase

interface. Figure 3.3 shows the different surface modifications and the liquid interface that the particles preferentially adsorb to.

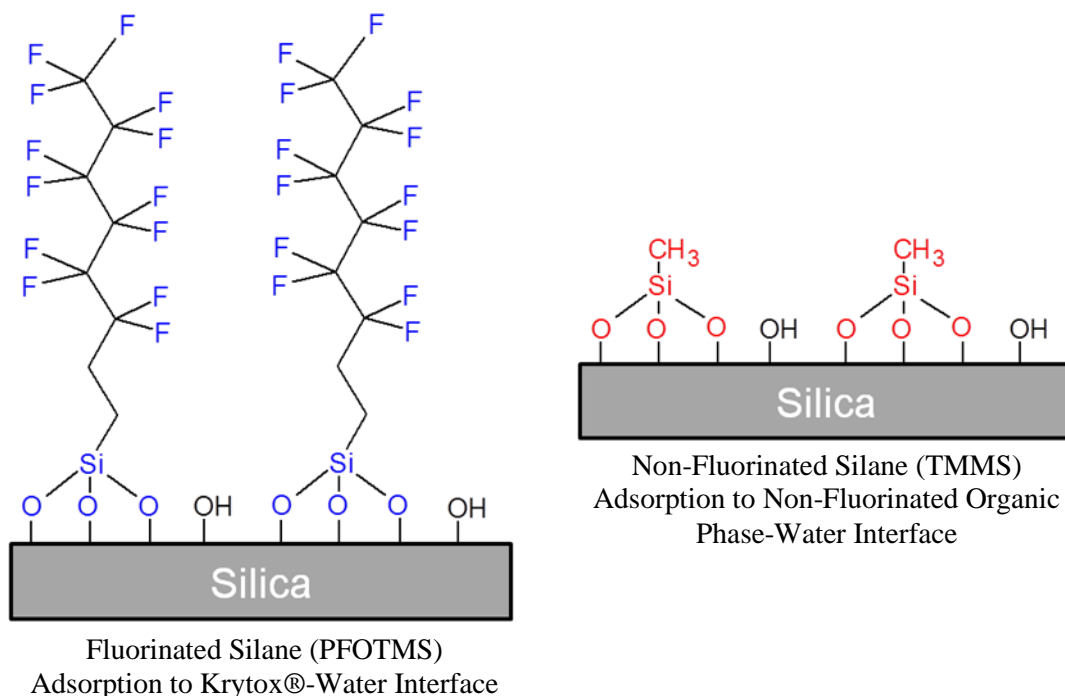


Figure 3.3: Depiction of surface modifications used and the desired interfaces of adsorption

With the combination of silica particles that can be modified to control their adsorption and three phase liquid systems with different starting droplet morphologies, experiments could then be performed to observe changes in droplet equilibrium structures due to particle adsorption.

3.4 Experimental Section

3.4.1 Phases and Interfacial Tensions

The interfacial tensions of the liquid phases were measured with a Rame Hart pendant drop tensiometer. The phases used were dodecane (SIGMA), hexadecane (SIGMA), tetradecane (SIGMA), toluene (SIGMA), Krytox® GPL001 (DuPont) and DI

water. The interfacial tensions were measured after the three phases of interest were combined, shaken by hand and allowed to equilibrate for 24 hours. Interfacial tensions that were measured in the presence of particles used a liquid phase that contained 0.5 wt% of the desired particles as the bulk liquid. The dynamic interfacial tension was then measured until it equilibrated. In the cases where DI water was used as a liquid phase, 10mM NaCl was added.

3.4.2 Particles and Particle Modification

Aerosil® 300 silica particles (EVONIK) were used for all experiments. The silica particles in their native state are too hydrophilic to adsorb to any interface. To enhance the interfacial adsorption of the particles, they were modified with either trimethoxymethylsilane (SIGMA), perfluorooctyltrimethoxy methylsilane (GELEST). To modify the particles, 1 gram of silica particles and a weight percent of the silane (weight percent of the silane is relative to the weight of particles) were added to 25 mL of toluene (SIGMA) and stirred for 24 to 48 hours. After the particles had been stirred, they were washed and centrifuged at 3000 rpm 3 times with fresh toluene to remove the non-reacted silane. The particles are then placed in an oven set to 110°C overnight. After the particles have been dried, they are ready for use.

3.4.3 Direct Droplet Experiments

Square glass capillaries (VITROCOM, 0.90 ID x 0.18 mm wall) were used in experiments where the droplets were directly contacted with each other. The square capillaries were prepped by cleaning them in piranha solution for an hour (3:1 ratio of H₂SO₄ to H₂O₂). Round capillaries (VITROCOM, 0.70 ID x 0.87 OD), that had been pulled to form sharp tips, were used with a syringe and tubing to inject small droplets into

the square capillary. In a typical experiment, water was used as the bulk phase. Due to the density difference between the two droplet phases, tilting the capillary so that the denser droplet moves towards the less dense droplet was sufficient in guiding the droplets to make contact and form an equilibrium structure. Once the initial equilibrium structure was observed, a solution of particles (0.1-0.5 wt% w 10mM NaCl) was injected into the square capillary. Care was taken to ensure that the droplet structure was not pushed out of the capillary when the particle solution was injected. Images were taken subsequently to observe the changes in droplet morphology due to particle adsorption.

3.5 Results and Discussion

3.5.1 Partial Engulfing Starting Point

Of the three possible equilibrium morphologies we observed, a good starting point was the partial engulfing morphology. Changing the morphology to any state from a partial engulfing starting point would simply require the negative spreading coefficient of one phase to change. The effective spreading coefficient (S_i^{\sim}), defined as the new spreading coefficient due to the adsorption of particles, of either the engulfing phase or the bulk phase would have to become positive due to the adsorption of particles to the appropriate interface. Figure 3.4 shows these two different approaches to demonstrating morphology change.

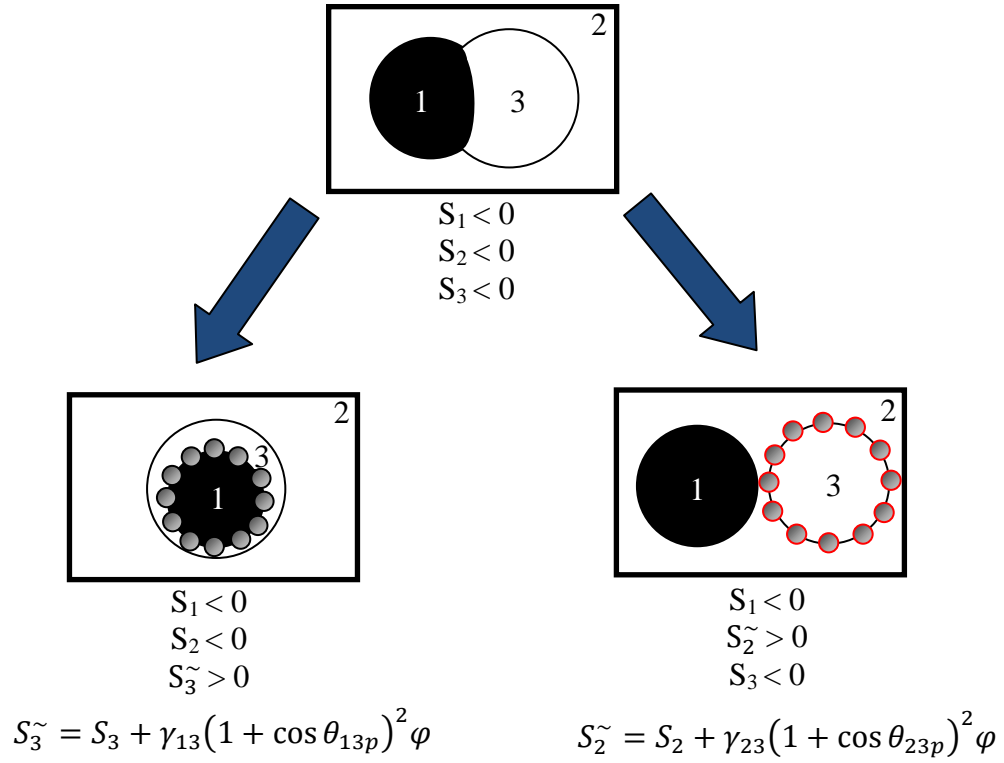


Figure 3.4: Depiction of morphology shift from partial engulfing due to particle adsorption at different interfaces

A three phase system that was selected for the demonstration of morphology change used Krytox®, DI Water and an alkane ranging from dodecane to hexadecane. Table 3.1 shows the interfacial tensions and spreading coefficients of this three phase system.

Table 3.1: Interfacial tensions and spreading coefficients of the Krytox®, DI water system with dodecane, tetradecane and hexadecane as the third phase

Phase 1	Phase 2	Phase 3	γ_{12} (mN/m)	γ_{13} (mN/m)	γ_{23} (mN/m)	S_1 (mN/m)	S_2 (mN/m)	S_3 (mN/m)
Krytox®	Water	Dodecane	55.5	7.83	50	-13.33	-97.67	-2.83
Krytox®	Water	Tetradecane	55.5	8.13	52	-11.63	-99.37	-4.63
Krytox®	Water	Hexadecane	55.5	8.78	51	-13.28	-97.72	-4.28

Since the alkane phase has the least negative spreading coefficient, it has the greatest tendency to spread. For direct droplet experiments it would therefore be ideal to have the alkane serve as either the engulfing phase or the bulk phase. While it is possible to use the alkane as the bulk phase, surface modification of the glass capillary is required to ensure that neither water nor Krytox® wet the capillary surface. It was much easier to simply use capillaries that had been cleaned with piranha solution to ensure that only water would wet the capillary surface. When water is used as the bulk liquid, the alkane will partially engulf Krytox®. The spreading coefficient of the alkane (S_3 in Table 3.1) ranges from -2.83 to -4.63 mN/m and can easily be overcome by particle adsorption. But this will require particles that can preferentially adsorb to the alkane-water interface with the appropriate contact angle and particle packing fraction.

3.5.2 The Complete Engulfing Starting Point

An alternative starting point, that was common among the three phase systems we observed, was the complete engulfing morphology. Shifting the morphology from complete to partial engulfing also requires only one spreading coefficient to change. Figure 3.5 depicts a scenario that would lead to a change from complete to partial engulfing. A three phase system that was selected for the demonstration of morphology change used Krytox®, DI Water and toluene. Table 3.2 shows the interfacial tensions and spreading coefficients of this three phase system.

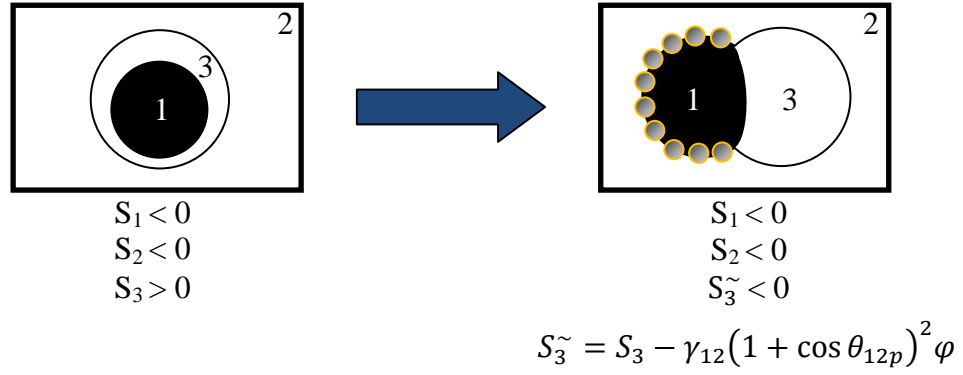


Figure 3.5: Depiction of morphology shift from complete engulfing to partial engulfing due to particle adsorption

Table 3.2: The interfacial tensions and spreading coefficients of Krytox®, DI water and toluene

Phase 1	Phase 2	Phase 3	γ_{12} (mN/m)	γ_{13} (mN/m)	γ_{23} (mN/m)	S_1 (mN/m)	S_2 (mN/m)	S_3 (mN/m)
Krytox®	Water	Toluene	55.5	8.0	32.3	-31.2	-79.8	15.2

The spreading coefficient of toluene (S_3 in Table 3.2) is 15.2. This spreading coefficient is a bit high to be significantly reduced by particles but it is definitely surmountable based on other work performed in our group [25]. The particles used in this case would have to preferentially adsorb to either the water-toluene interface or the Krytox®-water interface for a morphology change to occur.

3.5.3 Theoretical Predictions

In an attempt to get preliminary predictive information about the three phase systems that we were studying, the theory of particle assisted spreading developed by Goedel et al. was used [31]. The theory of particle assisted spreading uses bulk interfaces, similar to those used in Goedel's experiments, and calculates the equilibrium morphology

by determining the least energetic equilibrium state. Using Goedel's theory of particle-assisted spreading, a phase diagram was generated to qualitatively determine the probability that particles that preferentially adsorb will cause a shift in morphology. Figure 3.6 shows a phase diagram that has been generated in MATLAB using Goedel's theory for the three phase system containing Krytox®, DI water and an alkane.

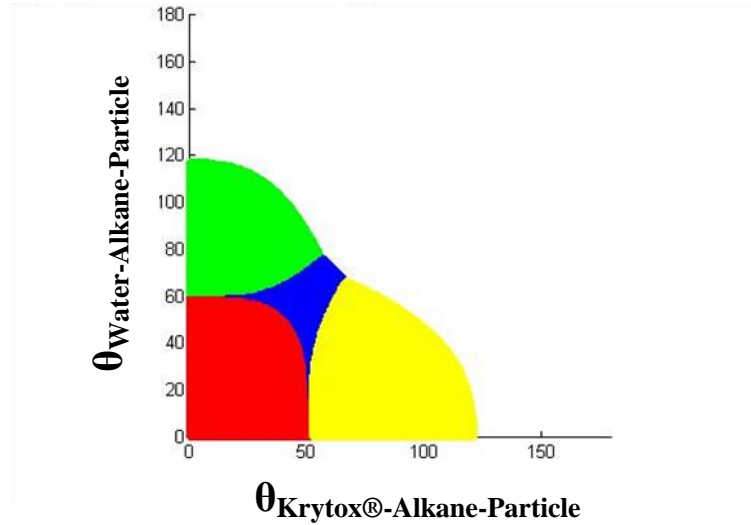


Figure 3.6: Phase diagram of a Krytox®-alkane -DI water system at maximum coverage

The regions in green, blue and yellow represent regions where spreading is possible. If it is desirable to shift the morphology of a droplet structure from partial to complete engulfing, particles that possess the contact angles in these regions would achieve that end. The regions in red and white are regions where spreading will not occur. If the particles possess contact angles in these regions, nothing will happen. Figure 3.6 displays the scenario when the particles are packed to the maximum packing fraction. In reality the particle fraction may not be as high and hence, the regions where spreading is possible will be smaller. Unfortunately, we are not able to easily measure the relevant model parameters of particle contact angle and particle packing fraction. Knowledge of

these parameters would make it possible to produce an accurate phase diagram and validate its applicability to a variety of three phase droplet structures.

3.5.4 Bulk Phase Experiments

In order to quickly screen three phase systems and test the impact of particle adsorption qualitatively, bulk phase experiments were performed. In these bulk experiments, two of the three immiscible liquid phases are placed in a glass cell. A droplet of the third phase is brought to the interface of the other two interfaces via the use of a syringe. Figure 3.7 shows a depiction of the setup used in this experiment.

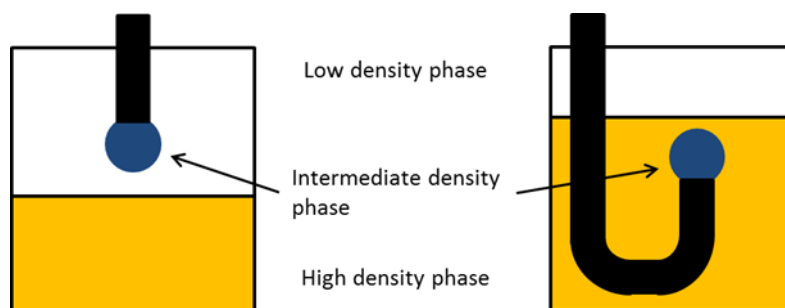


Figure 3.7: Depiction of bulk phase experimental setup

The results from the bulk phase experiments corresponded well with the predicted spreading behavior determined by the interfacial tensions and spreading coefficients.

Figure 3.8 shows examples of results from the experiment that demonstrate the three possible equilibrium morphologies. In the bulk experiments where particles were used, it was very clear when they made an impact on the droplet morphology. Figure 3.9 shows examples of bulk experiments where the impact of particles was observed.

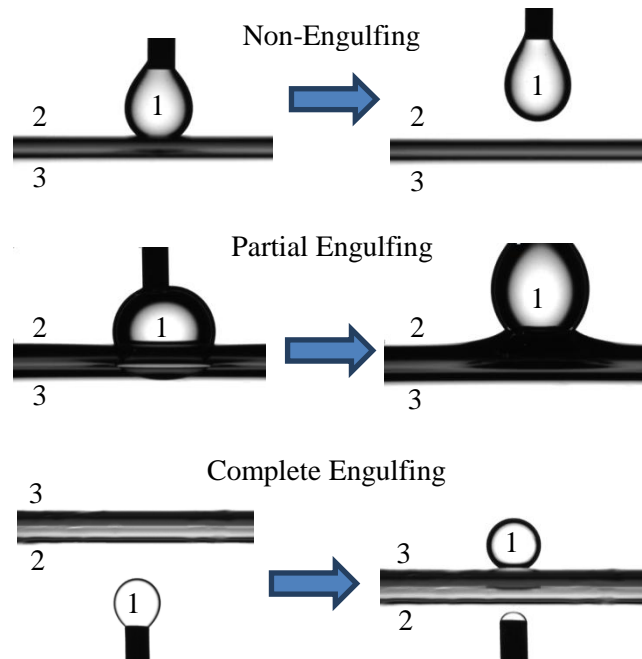


Figure 3.8: Images from bulk droplet experiments with different morphologies. Phases 1 & 3 correspond to the engulfed and engulfing phases respectively while phase 2 is the designated bulk phase.

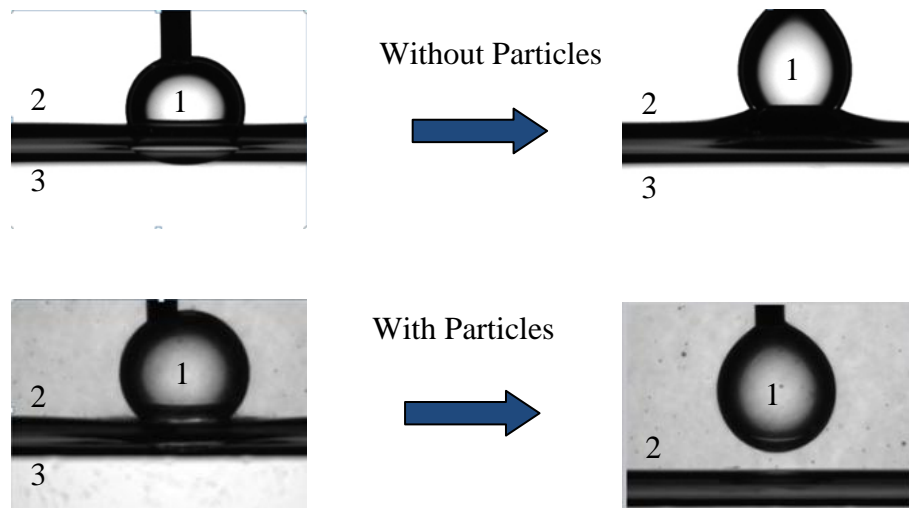


Figure 3.9: Images from bulk droplet experiments performed without particles (top) and with particles (bottom). Phases 1 & 3 correspond to the engulfed and engulfing phases respectively while phase 2 is the designated bulk phase.

The bottom images of Figure 3.9, in contrast to the top images, show that the presence of particles shifts the morphology from a partial engulfing morphology to an

apparent non-engulfing morphology. From the images shown, there is a clear difference in behavior due to the presence of particles. The bulk phase experiments were solely used for the purpose of quick analysis of three phase systems. It was still necessary to perform experiments involving small droplets brought into direct contact to make more conclusive deductions about the impact of particle adsorption in the investigated three phase systems.

3.5.5 Direct Droplet Experiments

To convincingly demonstrate changes in droplet morphology due to particle adsorption, small droplets of two of the immiscible liquid phases were brought together in the presence of the third phase. The Krytox®-dodecane-water system, which immediately forms a partially engulfed droplet, was used as the primary three phase system for direct droplet experiments. The first experiments using particles involved silica particles that were modified with TMMS. In the Krytox®-dodecane-water system, surface modification with TMMS would preferentially adsorb to the dodecane-water interface. Adsorption of TMMS modified particles to the dodecane-water interface would reduce the interfacial energy cost and ultimately increase the interfacial area. Figure 3.10 shows results from the direct droplet experiments both with and without particles.

The results from the experiment, shown in Figure 3.10, called into question whether or not the system was in a legitimate equilibrium state. The spreading coefficient of the water phase (S_2 in Table 3.1) is -97.67 mN/m and it is not feasible for particle adsorption to overcome a barrier to spreading of this magnitude.

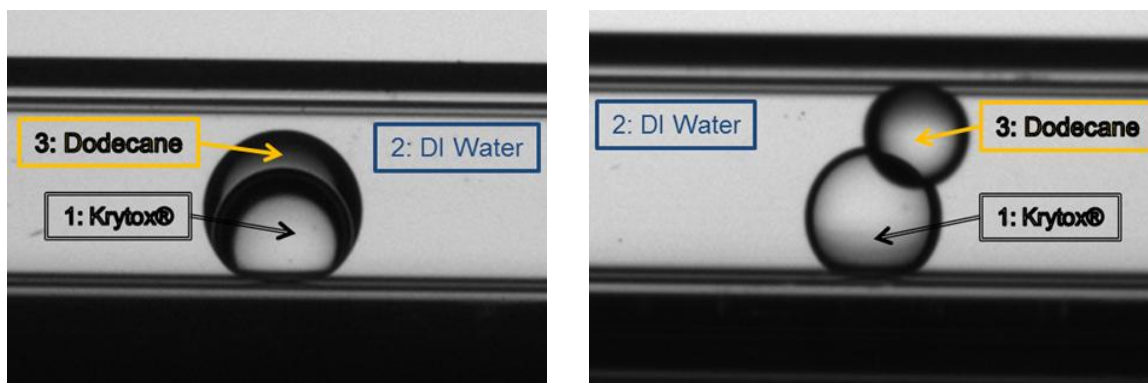


Figure 3.10: Direct droplet experiments performed with Krytox® and dodecane in water with particles that have been modified with 13 wt% TMMS.

The particles were added to the water phase before the droplets of Krytox® and dodecane made contact and even after 24 hours, the droplets maintained the same morphology. In order to test the validity of the initially observed equilibrium state, the same experiment was performed but the particles were added after the droplets had made contact. Figure 3.11 shows the result of adding particles after the droplets of Krytox® and dodecane made contact.

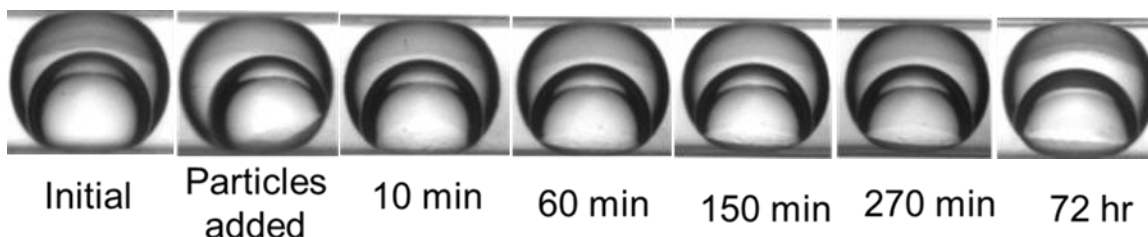


Figure 3.11: Direct droplet experiments performed with Krytox® and dodecane in water with particles that have been modified with 13 wt% TMMS.

Figure 3.11 clearly shows a different, yet more plausible, equilibrium state in comparison to Figure 3.10. After observing these results, it was apparent that the particles should be added after the droplets have made contact to make the argument for morphology shift much more convincing.

3.5.6 Dynamic Interfacial Tensions and Spreading Coefficients

It is difficult to measure parameters like the particle packing fraction and the particle contact angle. The methods used to determine the particle contact angle and packing fraction require the optical observation of molds made from the trapped particles [46, 47]. Over the course of this project, the discovery was made by our group that changes in interfacial tension due to particle adsorption, also called the effective interfacial tension, can accurately describe the changes in droplet morphology in air-oil-water system [25]. Measuring the effective interfacial tension makes it easy to quantify the impact of particle adsorption on the spreading coefficients and ultimately the total change in free energy.

3.5.7 The Krytox®-DI Water-Tetradecane System

The previous direct droplet experiments used TMMS as the surface modifier with seemingly minimal success. Surface modifications as high as 100 wt% TMMS were ineffective at substantially changing the resulting droplet morphology. At this point, surface modification was attempted with a fluorinated silane (PFOTMS) with the intention of adsorbing particles to the Krytox®-water interface. Figure 3.12 shows the result of direct droplet experiments using PFOTMS particles.

Figure 3.12 shows an obvious shift in droplet morphology over the course of about 3 hours. As soon as the particles are added, the Krytox®-water interfacial area very clearly increases. The morphology change is substantial, but not a clear shift from one type of morphology to the other as we had initially hoped to achieve. Due to the difficulty involved in creating droplets of a uniform size from experiment to experiment, the contacted droplets are not the same size.

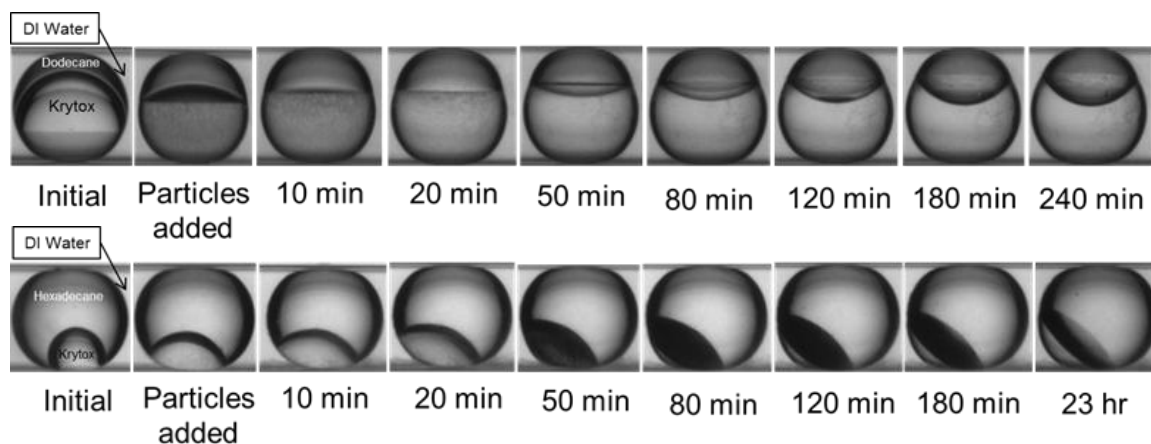


Figure 3.12: Direct droplet contact experiment performed with Krytox® and dodecane in water with PFOTMS modified particles

Figure 3.13 shows the measured interfacial tensions of the Krytox®-DI water-tetradecane system both before and after particle adsorption. The PFOTMS modified particles have the greatest impact on the Krytox®-DI water interfacial tension. This is expected due to the presence of fluorinated groups in the silane as well as in Krytox®. What is unexpected is the magnitude of change in the effective interfacial tension due to particle adsorption. A reduction in interfacial tension of 8 mN/m is not typical for silica particles. The surface modification is clearly responsible for the change.

Figure 3.14 shows the change in the effective spreading coefficient of Krytox® in the Krytox®-DI water-tetradecane three phase liquid system. The spreading coefficient of Krytox® increases from about -15 mN/m to -7 mN/m but does not ultimately become positive. If the spreading coefficient of Krytox® had become positive, the direct droplet experiments would have shown Krytox® completely engulfing the alkane in the presence of DI water.

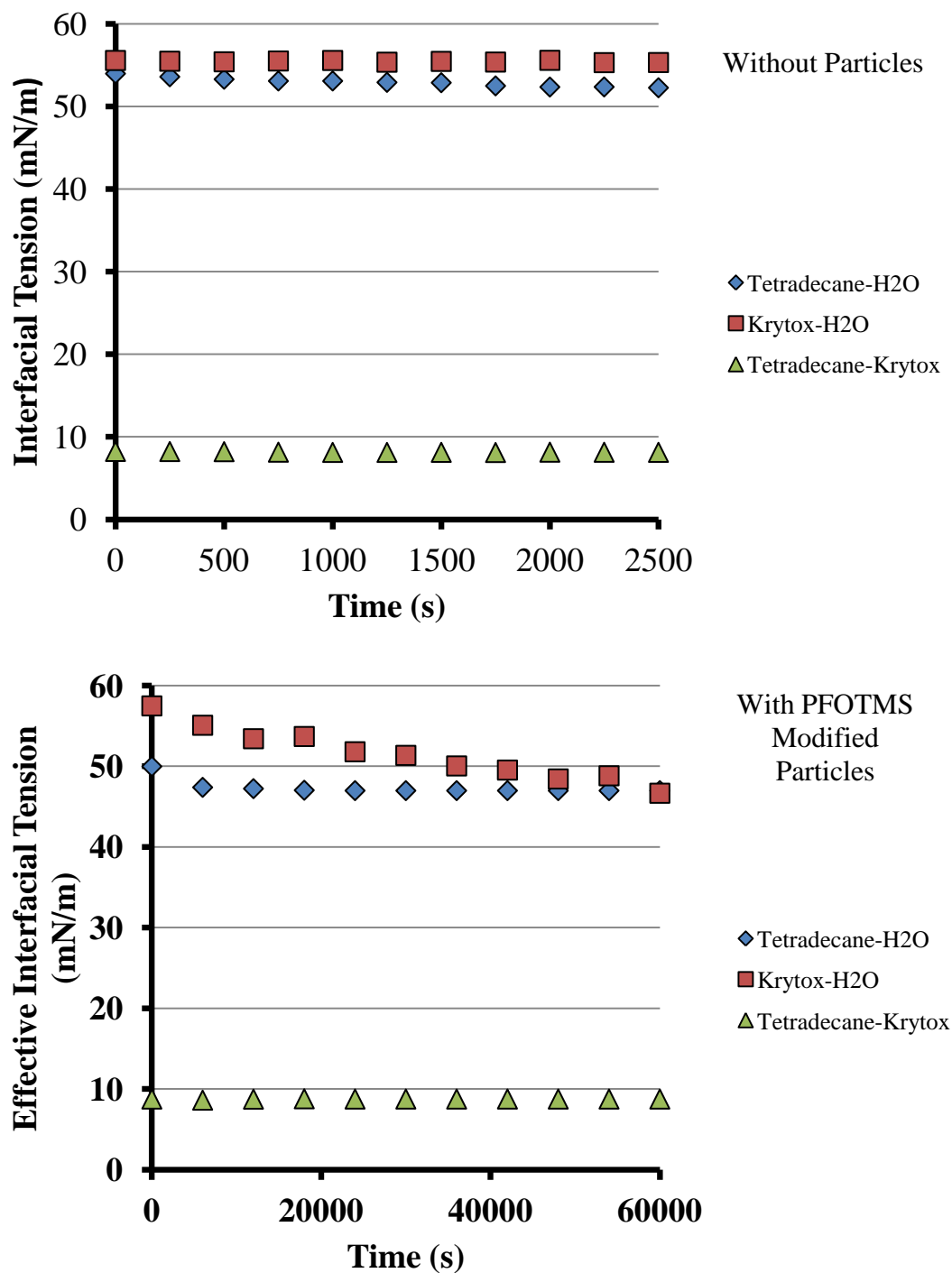


Figure 3.13: The interfacial tensions (top) and effective interfacial tensions of Krytox®, DI water and tetradecane in the presence of PFOTMS particles (bottom)

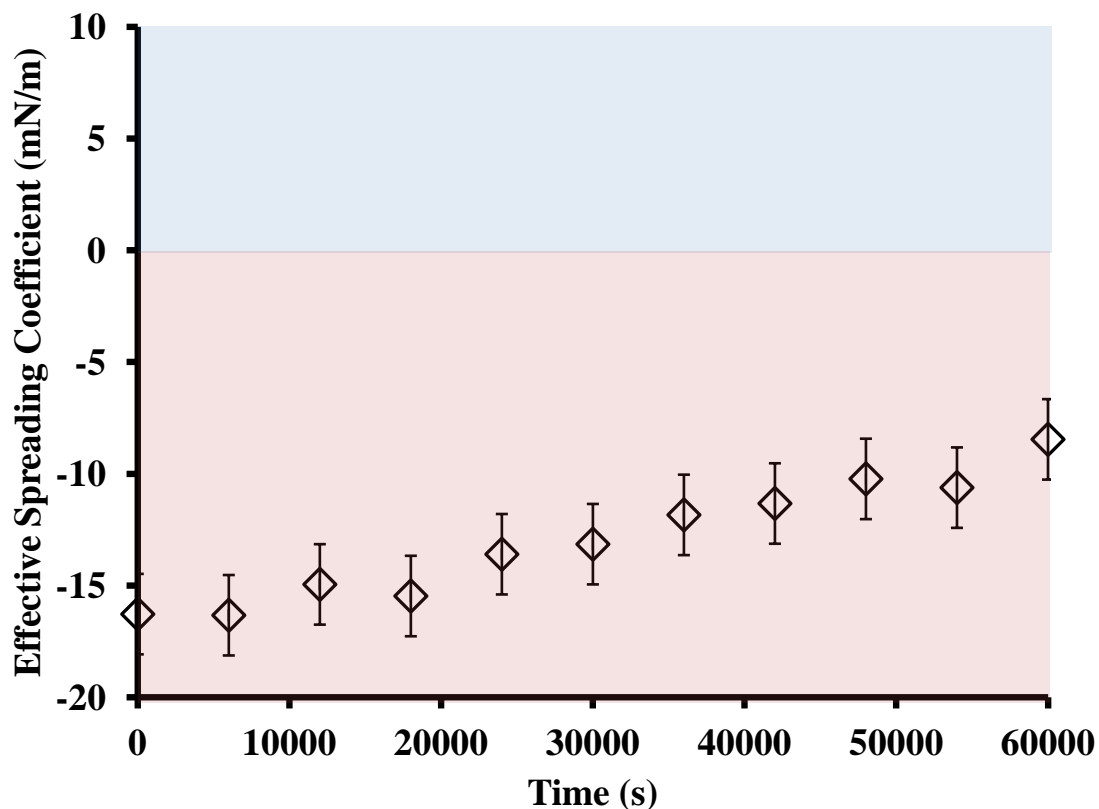


Figure 3.14: The effective spreading coefficient of Krytox®

3.5.8 The Krytox®-DI Water-Toluene System

In an attempt to demonstrate a clear change from one morphology to another, the Krytox®-toluene-DI water system was used. This three phase system initially forms a complete engulfing morphology which we believed could be shifted to a partial engulfing morphology with the addition of PFOTMS modified particles. Figure 3.15 shows the droplet experiment performed with this system. While it took much longer than the other direct droplet systems to reach equilibrium, the change in morphology is apparent. The increased length in time may simply be due to the fact that the Krytox®-water interface is not readily available for particle adsorption.

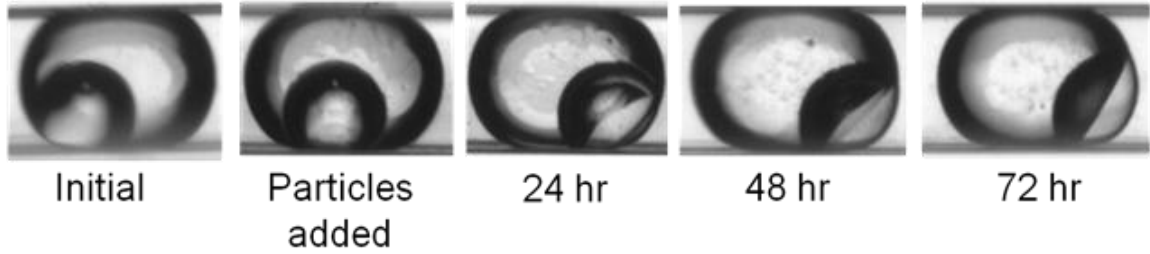


Figure 3.15: Direct droplet contact experiment performed with Krytox® and toluene in water with PFOTMS modified particles

Table 3.2 shows the measured interfacial tensions of the Krytox®-DI water-toluene system and Table 3.3 shows the tabulated values of the effective interfacial tensions after particle adsorption. Due to the length of time that it takes for the morphology of the Krytox®-DI water-toluene to change, measuring the dynamic interfacial tension in the presence of particles was impractical. The effective interfacial tension was measured after 24 hours, when the morphology has clearly changed from complete to partial engulfing.

Table 3.3: The effective interfacial tensions and effective spreading coefficients of Krytox®, DI water and toluene in the presence of PFOTMS modified particles

Phase 1	Phase 2	Phase 3	γ_{12} (mN/m)	γ_{13} (mN/m)	γ_{23} (mN/m)	S_1 (mN/m)	S_2 (mN/m)	S_3 (mN/m)
Krytox®	Water	Toluene	35.5	8.0	30	-13.5	-57.5	-2.5

Based on the results, it is plausible that the morphology can change from complete to partial engulfing. The effective interfacial tension of the Krytox®-water interface has been shown to reduce by about 20 mN/m at a maximum so far. It is still plausible for the effective interfacial tension to reduce further provided that the particle packing fraction and the contact angles are optimal. In the Krytox®-DI water-tetradecane

system, the reduction in the effective interfacial tension at the Krytox®-DI water interface was not as large as it was in the Krytox®-DI water-toluene system. Inconsistencies in the particle modification process may be to blame. Another explanation may lie in the differences in residual water solubility of the third phase; namely the alkane relative to toluene. Toluene has a greater residual water solubility than the alkanes that were used. Therefore the measured effective interfacial tensions obtained from the Krytox®-DI water-toluene system at the Krytox®-water interface are not necessarily comparable to the same effective interfacial tension measured in the Krytox®-DI water-alkane system. The fact that the three phase system is initially shaken and equilibrated for 24 hours makes it possible for residual toluene in the water phase to have an impact on particle adsorption. While the morphology change from complete to partial engulfing was not the desired one, it still demonstrates that particles are capable of significant morphology change. In order for changes in morphology to occur in other ways, particle modification that ensures preferential adsorption is required.

3.6 Conclusion

Whether the encounter of two immiscible liquid droplets in a liquid medium leads to a full engulfment of one drop by another, to partial engulfment, or to non-contacting droplets, is dictated by the interfacial tensions associated with each liquid interface. Surfactants have previously been used to shift the interfacial tension balance and change the observed liquid morphology. In this chapter, we have demonstrated that colloidal particles with suitable wettability can also be used to change the outcome of the droplet-droplet encounter. Although particles are known to provide an alternative to surfactants in the stabilization of emulsion droplets, the observed behavior is still remarkable. First,

particles are not generally known to have as big an impact on interfacial tension as surfactants do. Secondly, colloidal particles tend to adsorb irreversibly to a liquid interface, whereas surfactants adsorb only weakly and fluctuate in and out of the interface. As a consequence, one can rationalize that surfactants not only change the equilibrium morphology of two contacting droplets, but also allow the system to reach the energetically favored state. Colloidal particles, by contrast, could get “trapped in the wrong interface” and effectively stabilize non-equilibrium morphologies. For the systems investigated in this study, the particles adsorb to the desired interfaces due to the surface modifications that were performed. The equilibrium morphologies that result from the addition of particles can be explained by both Goedel’s theory of particle assisted spreading and by tracking dynamic changes of the interfacial tensions to calculate new effective spreading coefficients. The effective spreading coefficients correspond to the equilibrium structures that are observed and sufficiently explain the changes in droplet morphology. The following chapter describes how double emulsion templates can be used to produce novel capsules that are capable of extended release of an aqueous active ingredient.

CHAPTER 4

DEVELOPMENT OF NOVEL MICROCAPSULES

4.1 Background

Formulations of agrochemicals for crop protection often contain the active ingredient, a number of inert ingredients and adjuvants that improve storage, handling and facilitate uptake of the active ingredient by the plant. On the downside, however, common adjuvants can also compromise the stability of the formulation; for instance by promoting Ostwald ripening. It might therefore be beneficial to introduce in the formulation a spatial separation of the active ingredient from the adjuvants. Moreover, it is desirable in some cases to sustain the release of the active ingredient over a prolonged period of time, or more generally, to introduce a means of customizing the release profile.

4.2 Objective

The goal of the industrially sponsored project was to develop a novel method of microencapsulation for aqueous agrochemical formulations. The developed technique was expected to improve storage stability and stall release which was expected to improve the biological efficacy of the active ingredient upon application. This chapter will describe the new developments as well as the challenges that exist for the encapsulation of the specific dicamba containing active formulations, CLARITY® and ENGENTIA™, associated with this project.

4.3 General Approach & Rationale

The process of preparing oil soluble actives is much more straightforward than the encapsulation of water soluble actives. A common technique used in literature for the

encapsulation of oil soluble actives involves preparing an O/W emulsion template with the active dispersed in the oil phase. The oil phase can then be polymerized either entirely or interfacially and then easily separated from the water phase depending on the application. Within the scheme of published encapsulation techniques, there are few techniques applicable to the encapsulation of aqueous cargoes in aqueous environments. Previous work done in the Behrens group has provided the necessary experience in colloidal systems and emulsion technology to approach this problem [48, 49]. With this experience, the droplet-in-a-droplet morphology provided by W/O/W double emulsion was selected as the template for an aqueous phase encapsulation scheme.

While classical surfactants are more commonly used in the literature as double emulsion stabilizers, our double emulsions, in contrast, use colloidal particles to stabilize the droplet interfaces. Colloidal particles were used to improve the stability of the double emulsion droplets as well as to set a minimum thickness between the internal aqueous core of the W/O/W droplet and the aqueous medium which is depicted in Figure 4.1.

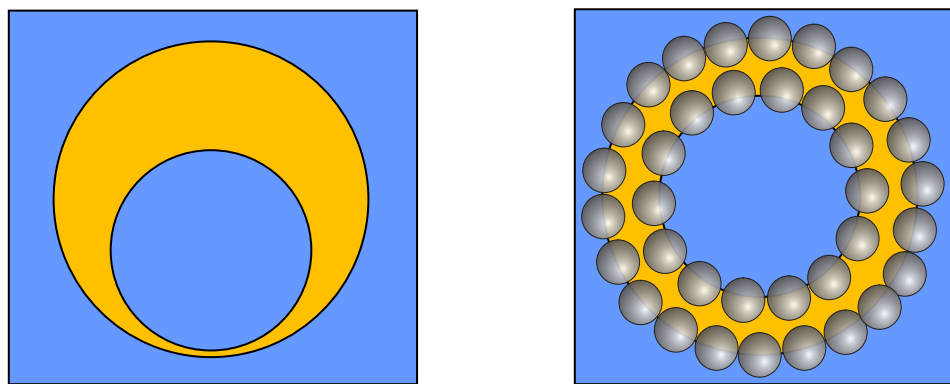


Figure 4.1: Illustration of a classical, surfactant stabilized (left) and a particle stabilized (right) W/O/W double emulsion

Work done by Goedel et al. has demonstrated that interfacially adsorbing particles can mediate the macroscopic spreading of oil into uniform films on water surfaces [26,

27], even in the case where the oil forms lenses in the absence of particles on the water surface. Because of Goedel's work, we hypothesized that a uniform oil layer would be able to surround the internal aqueous core provided that the particles had the appropriate wettability for the interface. The uniform oil layer would provide a greater diffusive barrier and aid in retarding the release of the aqueous active. The development of double emulsion droplets with a uniform oil layer was pursued as a separate part of the thesis and is discussed further in Chapter 2. Finally, we pursued the formation of polymeric capsule walls either by photo-polymerizing the oil phase or via interfacial polymerization at the O/W interfaces.

Our approach to encapsulating aqueous actives was constrained to ensure that the developed process was scalable. Double emulsions can be prepared using either batch techniques or on a droplet-by-droplet basis. In order to ensure uniformity, microfluidic devices are commonly used in academic studies to create double and multiple emulsions with immense control over the size, morphology and thickness of the middle phase [13-15]. A drawback associated with microfluidic devices is that they have a low throughput since flows on the order of $\mu\text{L}/\text{min}$ are used. In applications where small scale and high margin products are made that require monodisperse double emulsion droplets, microfluidic devices are a viable method of production. Whenever large quantities of double emulsion droplets are required, batch methods are preferred. The trade-off is that the droplets will not be monodisperse and there will be some variability in dosing on an individual capsule basis when they are prepared with conventional batch homogenizers. Since conventional batch homogenizers are more commonly used in industry and

scalable, we aimed to control morphology to the best extent possible using only batch homogenization techniques.

The project was defined by the achievement of a set of project milestones that if met would demonstrate the potential for this encapsulation method to be used in aqueous formulations of agrochemicals. The milestones are listed below:

- Milestone 1: Feasibility demonstration of W/O/W emulsion templates using particle-assisted oil spreading around the inner droplet phase.
- Milestone 2: Proof of concept for capsule formation.
- Milestone 3: Achievement of basic capsule properties:
 - Capsule sizes in the range of 1 - 50 μm
 - 10 % overall loading of a model active ingredient
 - Storage stability for 2 weeks at 0⁰C and 40⁰C.
- Milestone 4: Quantitative assessment of the technology's capability.

4.4 Experimental Section

Existing formulations of dicamba salts (dicamba-DGA, CLARITY® and, later, dicamba-BAPMA, ENGENIA™) were identified as a suitable models for an aqueous active ingredient to be encapsulated. In all capsule formulations CLARITY® or ENGENIA™ was used as the internal aqueous phase. The primary oil phase used was trimethylolpropane trimethacrylate (TMPTMA). This was for the ease of solidifying the oil phase to make capsules. Experiments were also performed with mineral oil, canola oil, paraffin oil, vegetable oil and dodecane as alternative oil phases. DI water was always used as the external aqueous phase.

4.4.1 Photo-polymerization of Double Emulsion Droplets

Due to the presence of a photo inhibitor (mono methyl ether hydroquinone) in TMPTMA, TMPTMA is first prepared by adding 1-2 wt% of aluminum oxide (SIGMA) and then stirring continuously for 24 hours. The mixture is then left to sit for another 12 hours to allow the aluminum oxide to sediment. TMPTMA is then decanted from the aluminum oxide and 0.1-0.5 wt% of hydrophobically modified silica particles, Aerosil® R805 (EVONIK), are added. Finally 0.1 wt% of benzoin isobutyl ether (TCI America) is added as a photo initiator. The external aqueous phase is prepared by simply adding 2 wt% of hydrophilic silica particles, Aerosil® 300 (EVONIK), to DI water.

0.75 mL of CLARITY® is added to 1.5 mL of the prepared TMPTMA oil phase and homogenized using a rotor-stator homogenizer (IKA Ultra Turrax T10) at 30,000 rpm for 30 seconds. This W/O emulsion is then poured into 3-5 mL of the external water phase. The mixture is then re-emulsified using a vortexer (Vortex Genie 2) for 30 seconds which completes preparation of the W/O/W double emulsion. The double emulsion is finally placed in a covered Petri dish and exposed to UV light (365 nm) for a period of 10 minutes. The UV Lamp used for photo-polymerization has a 100 watt bulb that produces a UV intensity of $27,000 \mu\text{W}/\text{cm}^2$ at a sample distance of 6 inch. After this time the double emulsion has been solidified.

4.4.2 Interfacial Polymerization of Double Emulsion Droplets

There are two primary encapsulation procedures for each respective aqueous dicamba formulation. The procedures detailed below are for standard formulations from which adjustments were made to change either release properties or the resulting capsule size.

4.4.2.1 CLARITY® Capsules

In the standard CLARITY® capsule formulation, the inner aqueous phase (W_1) was prepared by combining 0.675 mL of CLARITY® (an aqueous solution containing 324 mg of the active ingredient) with 0.075 mL of a 25 wt% aqueous solution of HMDA and vortexed for 10 seconds using a variable speed vortex mixer at the maximum speed setting. The oil phase (O) was prepared by dispersing the hydrophobic silica particles in 1.5 mL TMPTMA at a particle concentration of 2.0 wt%. The outer aqueous solution (W_2) was prepared by dispersing the hydrophilic silica particles in DI water (6.75 mL) at a particle concentration of 7.0 wt%. The inner solution (W_1) was emulsified in the oil phase (O) by homogenization for 10 seconds using a rotor-stator mixer set at 8000 rpm to prepare the inner (W_1/O) emulsion. Polymethylene diphenyldiisocyanate was added to the W_1/O emulsion at a molar HMDA:diisocyanate ratio of 1:1.2 to induce the formation of a polyurea skin at the W_1/O interface and the resulting emulsion was vortexed at maximum speed for 10 seconds. After 3 minutes, the same amount of diisocyanate was added again as reactant for the second polymerization and the sample was again vortexed for 10 seconds. Next, the W_1/O emulsion was added to the aqueous dispersion (W_2) and emulsified by vortexing at maximum speed for 2 minutes to produce the ($W_1/O/W_2$) double emulsion. To this emulsion 0.75 mL of an aqueous 25 wt% solution of glycerol was added and the emulsion was vortexed one more time for 10 seconds at the maximum setting to induce polymerization of the second polymer layer. After 12 hours at room temperature the formation of a polyurethane skin at the O/W_2 interface was complete.

4.4.2.2 ENGENIA™ Capsules

In the standard ENGENIA™ capsule formulation, the inner aqueous phase (W_1) was prepared by combining 0.6825 mL of ENGENIA™ (an aqueous solution containing 409.5 mg of the active ingredient) with 0.0675 mL of a 25wt% aqueous solution of BAPMA and vortexed for 10 seconds using a variable speed vortex mixer at the maximum speed setting. The oil phase (O) was prepared by dispersing the hydrophobic silica particles in 1.5 mL TMPTMA at a particle concentration of 2.0 wt%. The outer aqueous solution (W_2) was prepared by dispersing the hydrophilic silica particles in DI water (6.75 mL) at a particle concentration of 7.0 wt%. The inner solution (W_1) was emulsified in the oil phase (O) by homogenization for 10 seconds using a rotor-stator mixer set at 8000 rpm to prepare the inner (W_1/O) emulsion. Polymethylene diphenyldiisocyanate was added to the W_1/O emulsion at a molar BAPMA:diisocyanate ratio of 0.22:1 to induce the formation of a polyurea skin at the W_1/O interface and the resulting emulsion was vortexed at maximum speed for 10 seconds. After 3 minutes, the same amount of diisocyanate was added again as reactant for the second polymerization and the sample was again vortexed for 10 seconds. Next, the W_1/O emulsion was added to the aqueous dispersion (W_2) and emulsified by vortexing at maximum speed for 2 minutes to produce the ($W_1/O/W_2$) double emulsion. To this emulsion 0.75 mL of an aqueous 25 wt% solution of glycerol was added and the emulsion was vortexed one more time for 10 seconds at the maximum setting to induce polymerization of the second polymer layer. After 12 hours at room temperature the formation of a polyurethane skin at the O/W_2 interface was complete.

4.4.3 HPLC Quantification of Dicamba

A Shimadzu HPLC was setup in the lab for quantification of release of the active ingredient, dicamba, in capsule dispersions in water. Before performing any experiments, the mobile phase, internal standard and samples need to be prepared. The mobile phase was a 60/40 mixture of HPLC grade water and HPLC grade acetonitrile. An additional 0.1 vol% of ACS grade trifluoroacetic acid was added to the mobile phase mixture as well. The mobile phase was typically prepared in 1L batches. The internal standard is a 5 mg/mL prepared solution of 3,5 dichlorobenzoic acid diluted with acidic methanol (98 vol% HPLC grade methanol and 2 vol% ACS grade acetic acid). The internal standard was typically prepared in 100 mL batches. A typical sample for analysis contains 3mL of the internal standard, 3.75 mL of the capsule supernatant and 23.25 mL of acidic methanol. Before the sample is analyzed, it is passed through a 0.2 μ m syringe filter. Calibration standards were prepared using pure dicamba at 10, 20 and 30 mg. Each of these standards contained 5 mL of internal standard and 45 mL of acidic methanol. During the experiment, the mobile phase flow rate is 0.5 mL/min, the wavelength is 280 nm, the column temperature is 50°C and 5 μ L of sample are injected for analysis.

4.4.4 Two Week Storage Tests

Once the capsules are prepared they were first washed to quantify their encapsulation efficiency. They were then placed in either an oven set to 40°C or placed in a freezer. They were kept there for two weeks and then analyzed with HPLC for their release properties.

4.4.5 Surfactant Stabilized Capsules

The surfactant stabilized capsules were prepared using the CLARITY® capsule procedure detailed in Section 4.4.2.1. The only difference was the change in the emulsion stabilizer used at the inner W_1/O interface and/or the outer O/W_2 interface. The inner W_1/O interface was stabilized with either 2 wt% hydrophobic silica particles or 5 wt% of the hydrophobic surfactant Span 80 (sorbitan monooleate). The outer O/W_2 interface was stabilized with either 2 wt% hydrophilic silica or 2 wt% of a 3:1 mixture of Span 80 and Tween 80 (Polyoxyethylene (20) sorbitan monooleate) respectively.

4.5 Results and Discussion

The following results and discussion section is organized loosely along the project milestones and highlights the challenges and insights gained while the milestones were pursued.

4.5.1 Particle Stabilized W/O/W Emulsion Templates and Control of Droplet Morphology

The aqueous dicamba-DGA formulation CLARITY® was used primarily as the internal aqueous phase. Trimethylolpropane trimethacrylate (TMPTMA, shown in Figure 4.2) was initially chosen as the oil phase, because of its previous use in studies of particle-assisted wetting and spreading [26, 27]. DI water (with 0.1 mM NaCl to allow for a little bit of charge screening) was generally used as the continuous phase.

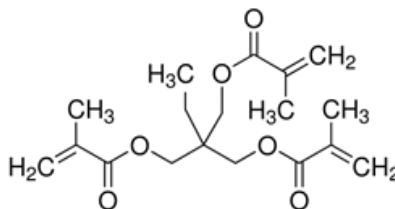


Figure 4.2: Chemical structure of TMPTMA

The traditional and most common method of producing W/O/W double emulsions consists of emulsifying water in an oil-based solution of a predominantly hydrophobic surfactant and then re-emulsifying the obtained oil-continuous emulsion in an aqueous medium with the help of a hydrophilic surfactant. This approach follows the century-old empirical Bancroft rule that for maximum emulsion stability the chosen emulsifier should be more soluble in the outer than in the inner emulsion phase [50]. The analogous rule for particle-stabilized emulsions demands that particles used for stabilizing W/O droplets be hydrophobic, while particles used for stabilizing O/W droplets be hydrophilic. Hydrophobicity and hydrophilicity are defined by a three phase contact angle measured through the water phase either above 90° or below 90° respectively. We decided to work with fumed silica particles, which were available to us in a range of different surface modifications (different degrees of hydrophobicity) and in sufficient quantities thanks to an earlier donation by the producer EVONIK. In initial W/O and O/W emulsification experiments, we identified the following two particle types, one hydrophobic (Aerosil® R805) and one hydrophilic (Aerosil® 300), as suitable for stabilizing the inner and outer interface of our W/O/W emulsions, respectively. The differences between these particles are highlighted in Table 4.1. Typically, up to 2 wt% of the hydrophobic particles were dispersed in the oil and up to 7 wt% of the hydrophilic particles in the outer water phase prior to emulsification in order to achieve good emulsion stability.

Table 4.1: Particles used for emulsion stabilization and capsule formation

Name	EVONIK Aerosil® R805	EVONIK Aerosil® 300
Particle Material	Hydrophobic fumed silica	Hydrophobic fumed silica
Surface modification	Octylsilane	none
Size of primary particles	12 nm	7 nm
Typical aggregate size	320-390 nm (average diameter in TMPTMA, similar in other tested oils, according to DLS)	210-250 nm in water

The use of surfactant additives was avoided due to prior studies on the synergistic effects of particles and surfactant emulsifiers. Even small amounts of surfactants can change the wettability of the particles when they adsorb to the particle surface and render them unsuitable for emulsion stabilization [51]. In addition, some surfactants can have negative environmental impacts if they are used in large amounts.

In order to produce W/O/W microcapsules that are uniform in their performance, the double emulsion droplets need to possess the same morphology. The desired W/O/W droplet morphology contains a large single drop of the active ingredient enclosed in the oil phase to ensure uniformity of the oil layer thickness and hence a predictable release rate. This is not the droplet morphology that results when the inner water phase in oil is homogenized and then emulsified again in an aqueous outer phase. First, we found that a milder agitation, or a lower shear rate, is generally required during the second emulsification step to avoid the predominant formation of single O/W emulsion droplets. When the liquid volume ratios and shear rates were adjusted to yield W/O/W droplets, the oil droplets usually contained a multitude of internal droplets, as seen below in Figure 4.3.

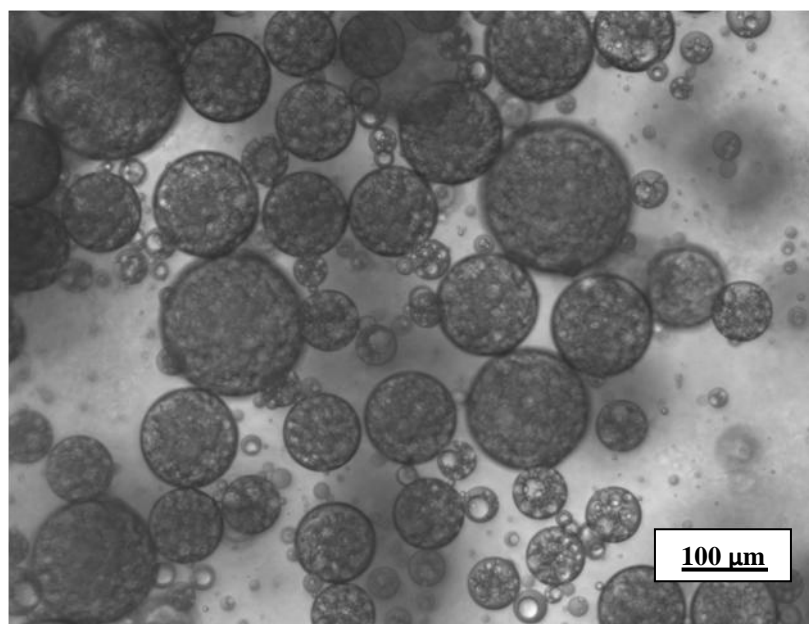


Figure 4.3: W/O/W double emulsion after homogenization. (Scale bar is 100 μm)

Since the W/O/W droplets observed in Figure 4.3 possessed many internal droplets, the possibility of high active loadings was put into question. We then decided to test alternative approaches that would produce droplets with single aqueous cores. We ultimately succeeded in achieving the desirable droplet morphology by using a combination of a reduced particle concentration and the addition of salt in the internal aqueous phase to induce osmotic swelling and coalescence of the internal aqueous droplets. This work led to a provisional patent being filed (“Water/oil/water emulsions including oil droplets containing a single aqueous core”, Patent application No. PCT/US2014/072654, Publication No. WO/2015/103190, Publication date: July 7, 2015, International filing date: December 30, 2014) and the details of this method are described further in Chapter 2.

4.5.2 Formation and Characterization of Emulsion Templated Solid Capsules

Our initial approach to making microcapsules from the templating of W/O/W emulsions consisted of photo-polymerizing the oil phase, TMPTMA. This would create a capsule that had a liquid core containing the active and a solid shell that would surround the liquid core. This method was initially tested by preparing an O/W emulsion with TMPTMA and stabilized with Aerosil® 300 particles. Figure 4.4 shows the TMPTMA droplets polymerized in solution and imaged after drying a dispersion drop on a glass substrate.

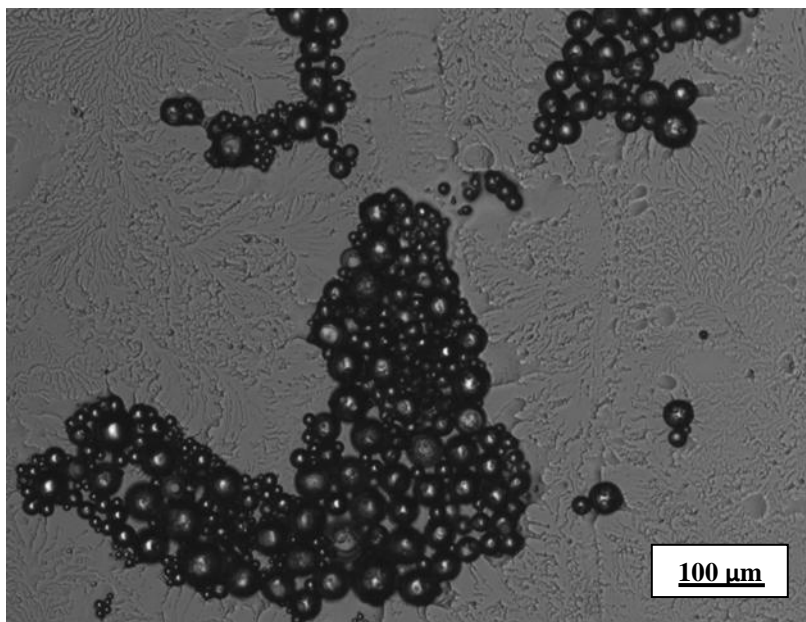


Figure 4.4: Polymerized TMPTMA droplets after 10 minutes of UV exposure. (Scale bar is 100 μm)

After this treatment, the droplets were clearly solid as could be verified by rubbing them against a solid surface and observing both the mechanical resistance and the shape retention expected for solid beads. The photo-polymerization procedure was then applied to a CLARITY®/TMPTMA/water double emulsion which is shown below in Figure 4.5.

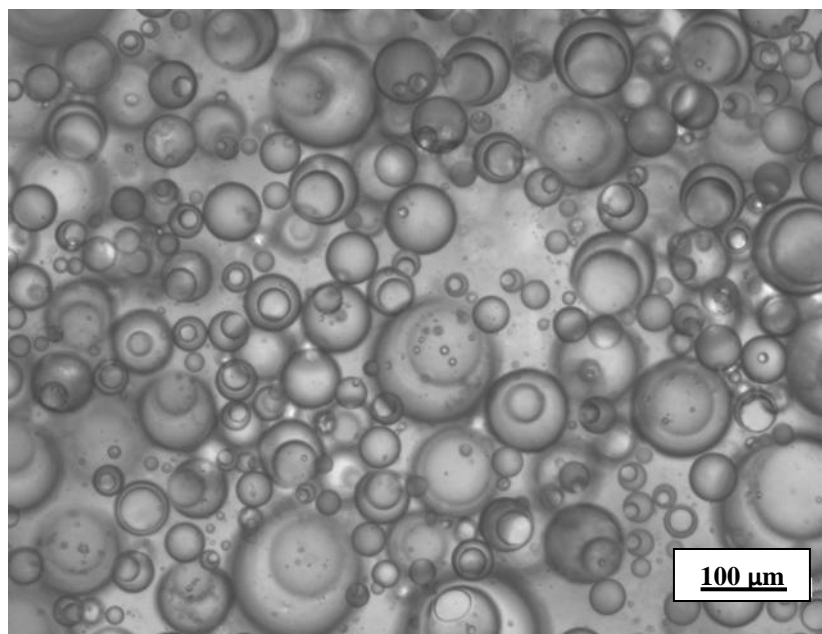


Figure 4.5: Photo-polymerized W/O/W double emulsion droplets that have been osmotically swollen and exposed to UV for 10 minutes (Scale bar is 100 μm)

This double emulsion had also been osmotically swollen before the photo-polymerization in order to produce capsules that had a single internal aqueous core. With an established method of making capsules, it was then important to quantitatively assess their release performance.

4.5.2.1 Quantification of Encapsulation Efficiency and Release

The encapsulation efficiency and active ingredient release in aqueous capsule dispersions was monitored using an HPLC setup specifically purchased and installed by our lab for this project. The method provided by the sponsors, detailed in the experimental section, was used to quantify the release of dicamba from a variety of capsule formulations. Figure 4.6 shows a typical HPLC chromatogram from a capsule release test using the aforementioned method. The first peak at 3.35 minutes is dicamba and the second peak at 5.216 minutes is the internal standard 3,5-dichlorobenzoic acid.

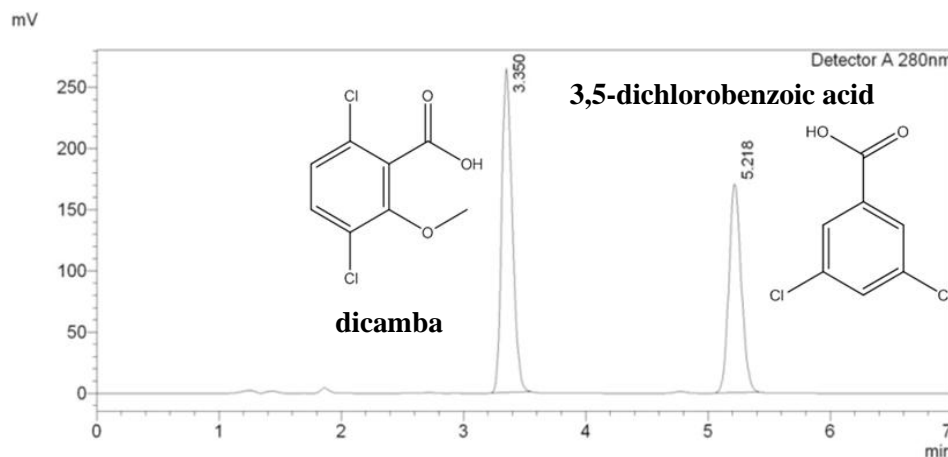


Figure 4.6: Typical HPLC chromatogram with the two relevant peaks for dicamba quantification

The encapsulation efficiency of dicamba in prepared capsules was determined by measuring the amount of it in the supernatant after centrifuging the freshly formed capsules at 4400 rpm for one minute. The amount found in the supernatant is subtracted from the total amount of dicamba originally loaded into the templating double emulsion. Finally, the difference is then divided (normalized) by the total amount of dicamba loaded to yield the encapsulation efficiency. Sustained release tests were performed over the course of 7 days. To perform these tests, the supernatant is replaced by fresh DI water after each analysis and stirred continuously for 24 hours. Each day, the cycle of centrifugation, supernatant replacement and HPLC analysis is repeated. This iterative process is depicted in Figure 4.7. After about 7 days, the release from the capsules typically falls below the detection limit of the HPLC detector. For this reason, the tests were only performed for 7 days.

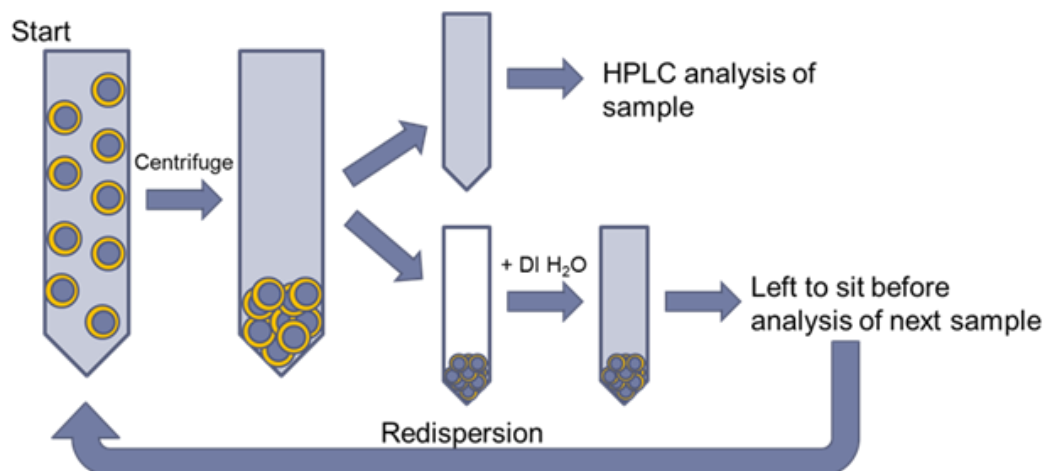


Figure 4.7: Depiction of the capsule analysis procedure repeated every 24 hours

The results from the release studies we performed give us a result associated with a specific set of conditions: the release of the active ingredient from the capsules due to diffusion and convection with the highest possible concentration gradient re-established every 24 hrs. Since we are designing capsules that are intended for extended release applications, it is not feasible to test them over the extended time periods they are designed for and have a reasonable amount of time to adjust their release properties. Before actual biological tests were performed on actual weeds in realistic conditions by the industrial sponsor, the aforementioned “accelerated release test” was used as a method of determining which capsule formulations were promising.

The photo-polymerized capsules unfortunately did not perform very well when their release behavior was analyzed. With these capsules, their encapsulation efficiencies typically ranged from 20-50%. It is likely that the high temperatures associated with the UV light could increase the diffusivity of the active before polymerization has been completed. Encapsulation efficiencies of 50% or below were not deemed viable or

practical for making capsules. At this point, an alternative methodology was developed to make capsules that had higher encapsulation efficiencies.

4.5.2.2 Capsules Formed by Interfacial Polymerization

The large amount of un-encapsulated active ingredient released in photo-polymerized capsules was ascertained to occur during the solidification step and during the second emulsification step. Since the inner droplet phase of the double emulsion droplet contains the entirety of the loaded active ingredient, it seems logical to polymerize the W/O interface between the inner droplet phase and the surrounding oil phase. This step would very likely increase encapsulation efficiency. Additionally, polymerizing the outer oil-water interface would provide a means by which the release of the active could be further stalled with an additional barrier to diffusion.

A protocol, provided by the industrial sponsor, already existed for encapsulating oil-soluble active ingredients in oil-in-water droplets with a polyurea shell formed by interfacial polymerization. O/W interfacial polymerization was achieved by first adding polymeric methylene diphenyl diisocyanate (PMDI, LUPRANATE® M20) to the oil phase before emulsification. Once the emulsion is prepared, a polyamine (hexamethylenediamine, HMDA) is delivered from the aqueous phase at a stoichiometric ratio of 1:1. Alternatively, the PMDI can be reacted with polyols in the water phase to form interfacial polyurethane. We adapted the existing protocols for O/W encapsulation to the inverse case of W/O droplets by adding HMDA (for polyurea) or glycerol (for polyurethane) to the dicamba solution prior to emulsification in oil, and PMDI to the continuous oil phase after the first (and before the second) emulsification step.

Solely polymerizing the outer O/W interface of the double emulsion droplets only marginally improved the encapsulation efficiency. The most promising procedure, with an encapsulation efficiency of 88-94%, was one in which polyurea was formed at the inner and polyurethane at the outer interface. Figure 4.8 shows a schematic of these capsules as well as images of the capsules and the different polymerization layers formed.

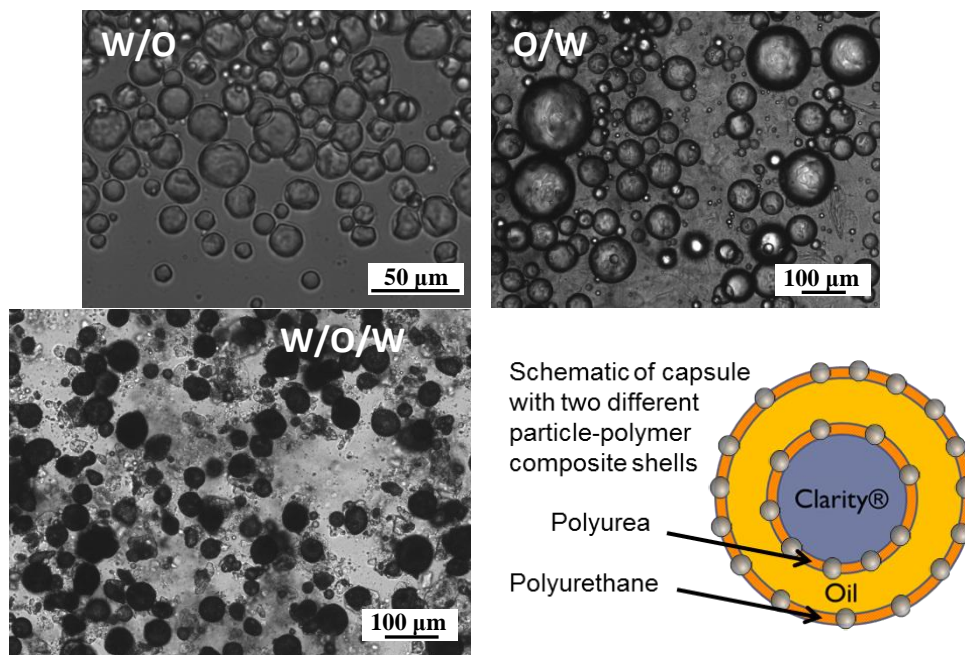


Figure 4.8: Interfacially polymerized emulsion droplets. Encapsulated single emulsion droplets of the W/O type (top left), O/W type (top right) and complete W/O/W emulsion based capsules (bottom) with a schematic of their double shell structure. While only one inner capsule is shown in the schematic for better clarity, capsules typically contain more than one inner capsule.

The comparatively high encapsulation efficiency for this type of capsule can be rationalized by noting that the reactivity of isocyanate with amines is far higher than with alcohols [52]. While interfacial polyurea formation proceeds to completion within seconds at room temperature and is known to yield polymer films of high elasticity and tensile strength, polyurethane formation takes several hours to complete and results in harder films. It appears that the fast-forming, elastic polyurea shells allow less time for

the inner emulsion to destabilize offering better protection of the enclosed water droplets during the second emulsification step. The slow-forming, but sturdy polyurethane shell assembled around the fully formed double emulsion droplet with an already encapsulated core droplet, adds to the overall mechanical stability of the capsule. Due to the utility and novel nature of the developed method of encapsulation, a joint patent application (“Microencapsulated water/oil/water emulsions having high encapsulation efficiency”, Patent application No. PCT/US2014/072659, Publication No. WO/2015/103195, Publication date: July 7, 2015, International filing date: December 30, 2014) was filed. While the strategy of encapsulating the aqueous inner droplets prior to the second emulsification clearly improved the encapsulation efficiency, it removed the option of the previously described formation of a single aqueous core by osmotically induced inner droplet coalescence (detailed in Chapter 2). As one might expect, the polyurea shell protecting the core droplets either prevents their swelling and coalescence or it ruptures and loses its protective function.

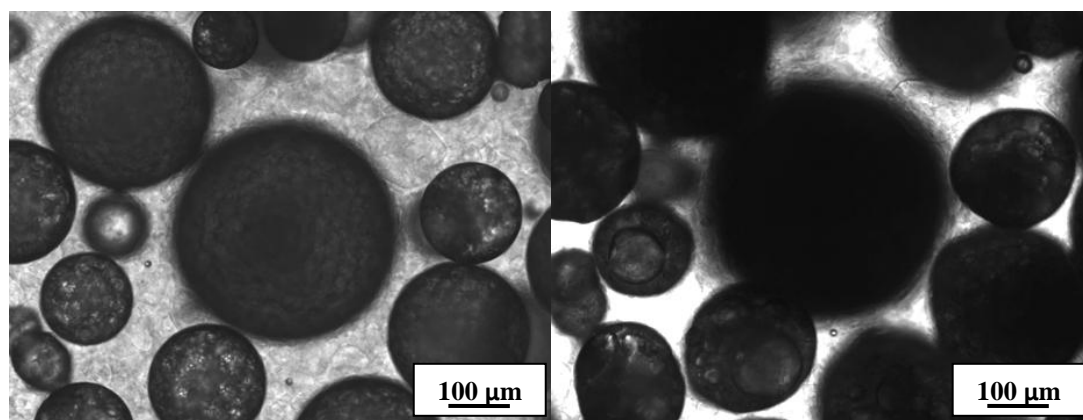


Figure 4.9: W/O/W capsules prepared with interfacial polymerization and osmotic swelling before (left) and after (right) swelling. Image quality is poor due to the polymerization of the interfaces (Scale bar is 100 μm)

Even the use of a polyol, to slowly polymerize the inner W/O interface, did not sufficiently allow for coalescence of the internal droplets to occur. Figure 4.9 shows capsules prepared with a polyol in the internal aqueous phase and osmotically swollen as much as possible.

4.5.2.3 Benefits of the Particles Embedded in the Capsule Shells

It has been mentioned previously that surfactants, when compared to Pickering particles, are more commonly used commercially as emulsion stabilizers. Given this fact, it was important to determine if there is a quantifiable benefit to our methodology of making capsules, using double emulsion templates stabilized by particles. To verify whether the presence of particles at the capsule interfaces provide a practical benefit, we compared encapsulated particle-stabilized double emulsion and encapsulated surfactant-stabilized double emulsions with regard to the encapsulation efficiency. The surfactant stabilizers used for this comparative experiment, Span 80 (sorbitan monooleate) and Tween 80 (Polyoxyethylene (20)), were chosen because earlier studies described in the literature had produced double emulsions with a droplet size and morphology that closely resembled our particle stabilized double emulsions [34]. Figure 4.10 shows our double emulsions containing a similarly large amount of internal aqueous droplets as the double emulsion from the literature.

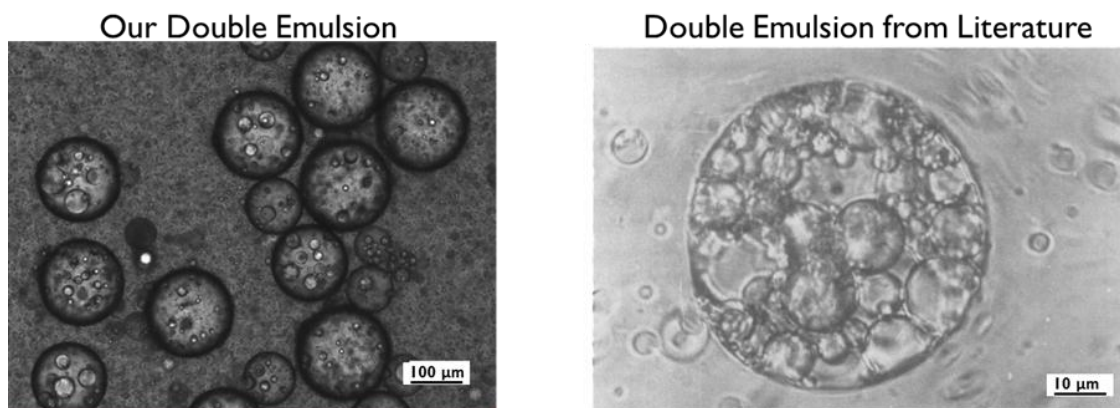


Figure 4.10: Our surfactant stabilized double emulsion (left) in comparison to the literature reference (Image on the right copied from [34])

Although the original double emulsion described in the literature contained pure water as the core phase and isopropyl myristate as the oil phase, a surfactant-stabilized analog of our double emulsions could still be formed and used as a reference capsule template. The double emulsions used for these tests contained CLARITY® as the active and TMPTMA as the oil phase. The double emulsion droplets were then interfacially polymerized using HMDA as the internal aqueous phase monomer, the isocyanate (LUPRANATE® M20) in the oil phase and glycerol as the external aqueous phase monomer. The results of these encapsulation efficiency experiments are summarized in Table 4.2. The data in Table 4.2 shows a very clear benefit of utilizing particles to stabilize the double emulsion interfaces. The system stabilized solely by particles outperforms all of the other capsules in regards to encapsulation efficiency. Replacing the surfactant by the particulate stabilizer at any interface increases the encapsulation efficiency. The biggest improvement to encapsulation efficiency is observed when the particles are used instead of surfactants at the inner W/O interface. This is consistent with our previous conclusion that the early loss of active primarily occurs during the second

emulsification step and can be remedied by firmly encapsulating the inner droplets in a protective shell before proceeding with the O/W emulsification.

Table 4.2: Encapsulation efficiencies of CLARITY® capsules prepared with surfactant and/or particles

Stabilizer of the W/O Interface	Stabilizer of the O/W Interface	Encapsulation Efficiency
Particles	Particles	88-94%
Particles	Surfactant	79%
Surfactant	Particles	49%
Surfactant	Surfactant	43%

A possible explanation for the increased encapsulation efficiency is the formation of a particle-polymer composite shell at the capsule wall. If true, the increased encapsulation efficiency indicates that the presence of particles in the composite shells created at the particle-stabilized interfaces truly benefit the active retention. In either case, the particles used for emulsion stabilization would become embedded in the capsule wall. In so-called “mixed matrix membranes” used for gas separations, particles embedded in a polymer skin are used to improve the barrier properties of membrane [53].

4.5.2.4 Alternative Oil Phases

Most of our encapsulation experiments used TMPTMA as the oil phase. TMPTMA is a convenient oil phase to use for photo-polymerization, but it may not be the most economic or environmentally friendly option. Because of these considerations, alternative oils were tested to observe their impact on encapsulation efficiency and, hence, viability for use with the developed encapsulation scheme. Both mineral and

vegetable oils have been shown to enhance herbicide absorption as well as retention [54]. Because of these expected benefits, mineral, paraffin, vegetable, and canola oils were tested. Canola and vegetable oil were chosen more specifically for their low ecological toxicity effects. Dodecane was also tested to observe if an alkane can also be effective as an oil phase alternative. Table 4.3 shows the results of this comparison experiment.

Table 4.3: Encapsulation efficiencies, densities and viscosities of oil phases used for interfacially polymerized capsules

Oil	Encapsulation Efficiency	Density (g/mL)	Viscosity (mPa*s)
TMPTMA	88% (minimum)	1.065	69
mineral oil	74%	0.83	10-13
canola oil	70%	0.92	57
paraffin oil	68%	0.8-0.9	190
vegetable oil	63%	0.9-0.93	65
dodecane	47%	0.7487	1.34

The results show that TMPTMA, the most polar oil tested, performed best with respect to encapsulation efficiency, while dodecane, the least polar oil, performed the worst. These oil phases differ in their density and viscosity, and although these parameters might be expected to matter, no systematic correlation with the encapsulation efficiency is observed. The most likely explanation for the difference in performance lies in the oil polarity and the particles that were used to stabilize the interfaces. (All of these samples contained the same silica particles as emulsion stabilizers). It is plausible that the particles that are optimal for stabilizing TMPTMA interfaces will not be as effective at stabilizing other oil interfaces. Particle surface modification, via silanization of silica

particles, is a method that can be used to adjust the particle contact angles and improve the stability of the capsules which could improve the encapsulation efficiencies with the other oil phases. While alternative oil phase considerations are important and relevant to the end use of the capsules, we decided to focus on meeting the required capsule parameters using TMPTMA due to its high encapsulation efficiency.

4.5.3 Basic Formulation Requirements

4.5.3.1 Capsule Size of 1-50 μm

Of the targeted basic capsule properties, meeting the specified upper limit for the capsule size presented the biggest challenge. This size criterion is important because it ensures that when the capsule dispersions are applied to the field, the spray nozzles will not clog and prompt the farmers to unclog the nozzles before application again. The main strategies pursued to reduce the initially large size of individual capsules involved homogenization at higher rotor frequency and/or the addition of a thickener to the continuous emulsion phase to enhance the shear forces transmitted at a given frequency. The initial W/O emulsion can easily be reduced to below 50 μm as can be seen in Figure 4.11. Size reduction of the initial W/O emulsion to 50 μm or less can be achieved solely by vortexing. In order to ensure that the resulting double emulsion droplets are as small as possible, the initial W/O droplets were reduced in size with a rotor-stator homogenizer to provide maximum shear. The biggest challenge of size reduction manifests during the second emulsification step. Using a rotor-stator homogenizer during the second emulsification step excessively shears the droplets and causes them to rupture. For this reason, the second emulsification step was done with a vortexer (Vortex Genie 2; Scientific Industries) to reduce the shearing intensity. However, even at the maximum

vortexer setting, the droplets only reduce to about 100 μm . In other words, homogenization via the rotor-stator mixer provides too much shear at the lowest setting while vortexing does not provide enough shear even at the highest setting. In order to increase the shear intensity during vortexing, a thickener, Xanthan gum, was added to the external aqueous phase. Initial tests with the thickener on the W/O/W capsules indicated that Xanthan Gum was required at 0.1 wt% to reduce the size adequately. Figure 4.12 shows how much size reduction was achieved by adding 0.1 wt% of Xanthan gum to the external aqueous phase.

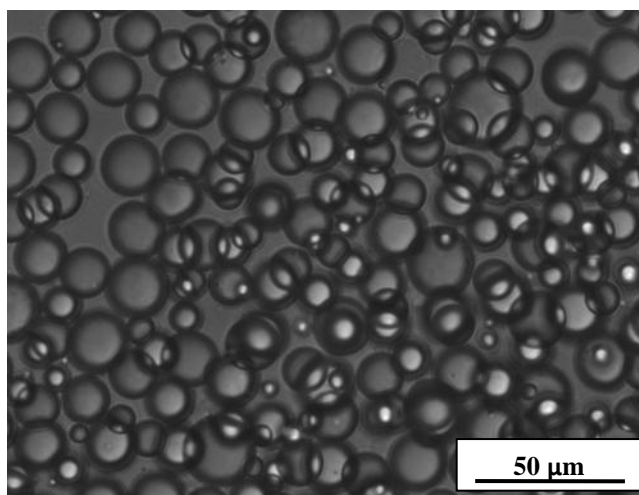


Figure 4.11: W/O emulsion of ENGENIA™ in TMPTMA (Scale bar is 50 μm)

It seemed intuitive that adding more thickener to the external aqueous phase would shear the droplets further. However greater weight percents of the thickener either make the external aqueous phase too thick, rendering size reduction via vortexing ineffective, or the droplets rupture and release their contents due to excessive shear.

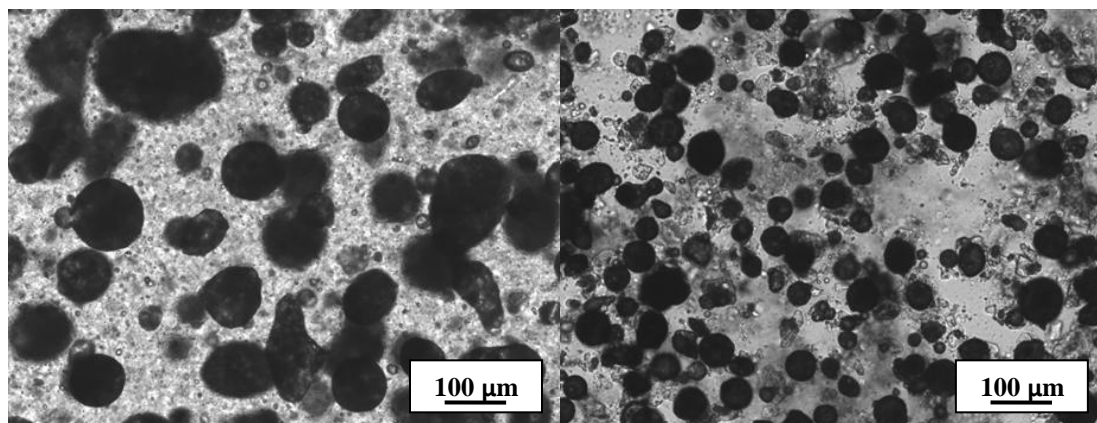


Figure 4.12: W/O/W interfacial capsules prepared without Xanthan gum (left) and with 0.1 wt% Xanthan gum (right) in the external aqueous phase (Scale bar is 100 μm)

Neither of these approaches, homogenizing the initial W/O emulsion and the use of thickener, when applied separately, succeeded in limiting the capsule size without causing an unacceptable active loss. The best results were obtained by combining both methods but applying them in different emulsification steps. First, homogenization with a rotor-stator mixer (UltraTurrax IKA T10) at 8000 rpm was used to reduce the maximum size of W/O droplets to 30 μm , as seen in Figure 4.11. Secondly, between 0.05 and 0.075 wt% of the thickener Xanthan gum was added to the external aqueous phase which made it possible to reduce the maximum double emulsion droplet size to 50 μm . Figure 4.13 shows capsules that have been reduced to the desired size range of 50 μm or less. While the desired size criterion has been met, it may still be necessary to further reduce the capsule's size. This will require optimization of the amount of thickener added to the external aqueous phase and of the types of particles added to in the outer water phase as stabilizers for the O/W interface. These consideration are important because their mutual interaction in the outer phase contributes to the viscosity and thus to the energy transferred to the droplets.

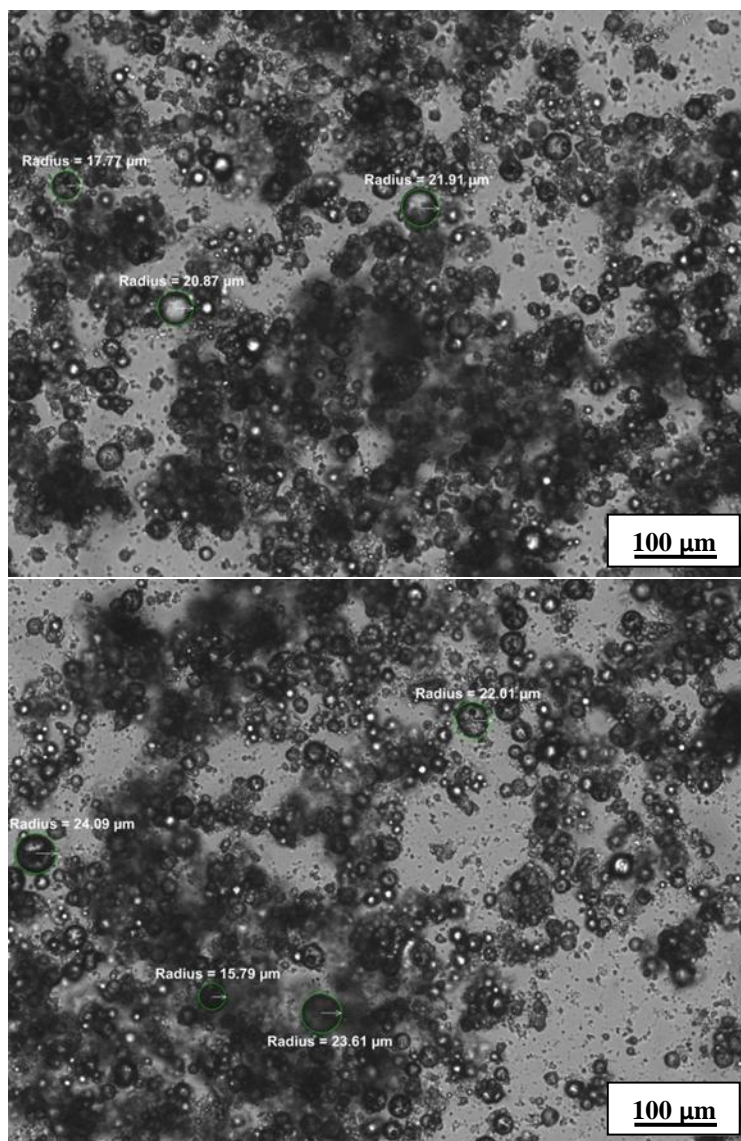


Figure 4.13: ENGENIA™/TMPTMA/water capsules (Scale bar is 100 μm)

In general, emulsion droplets will most readily be separated into smaller droplets by shear if the droplet viscosity is low and the continuous phase viscosity is high. It turns out that in our system the bulk viscosity of the oil phase also increases as the polyurea formation in the W/O emulsion proceeds. This thickening of the oil phase proceeds at different rates depending on the type and concentration of the amine monomer in the

aqueous droplet. If the oil phase thickens too rapidly, the added thickener will be ineffective and unable to sufficiently reduce the size of the droplets. The increased bulk viscosity of the oil phase creates a lower limit of capsule size that makes it difficult to keep the maximum overall size of the W/O/W droplets below 50 μm .

We had already succeeded in meeting the capsule size requirements for CLARITY® capsules with HMDA as the internal aqueous phase monomer, when we became aware of issues with dicamba volatility in this formulation (described in more detail in Section 4.5.4.4). Subsequent experiments used dicamba-BAPMA (ENGENIA™) and BAPMA as the internal aqueous phase monomer in an attempt to avoid any counter-ion exchanges that would raise the volatility of dicamba. Unfortunately, this replacement active formulation also led to a rapid increase in the oil phase viscosity upon initiation of the polyurea formation by addition of the isocyanate to the continuous oil phase of the inner emulsion. During the second emulsification step, thickening of the oil phase at a rapid rate can negate the size reducing benefit that the thickener provides. Successful size reduction to the sub 50 μm range, in the case of ENGENIA™ capsules containing additional BAPMA, required re-engaging the challenge of achieving sufficiently small oil droplets during the second emulsification step. Even with thickener concentrations above 1 wt% in the outer aqueous phase we were unable to reduce the capsules to 50 μm or less for capsules containing BAPMA both as counter-ion in the dicamba salt and as reactant for the isocyanate at the stoichiometric concentration ratio. A reduction in BAPMA concentration solves the problem of overall droplet and capsule size, but this reduction also affects dicamba volatility and the thickness/performance of the polyurea shell adversely. Further optimization may well be

possible, but the complex connection between dicamba volatility, capsule size and capsule thickness was understood too late in the project to complete the optimization within the project duration.

4.5.3.1.1 Re-dispersibility

After the capsules have been prepared and dried for storage and shipment, it is important that they can be easily re-dispersed completely in water. Even if the capsules have been reduced to the desired size range, ineffective re-dispersion will lead to the presence of large aggregates of capsules that will clog the spray nozzles. Originally our capsules were typically dried at room temperature in a fume hood for about 24 hours. After a day, they appeared reasonably dry and could be dispersed in water when they were ready to be used. More complete drying via mild heating or longer drying times, however, resulted in capsule aggregates that could not be fully re-dispersed. Figure 4.14 shows an example of a large aggregate of capsules that can result using this method of preparation.

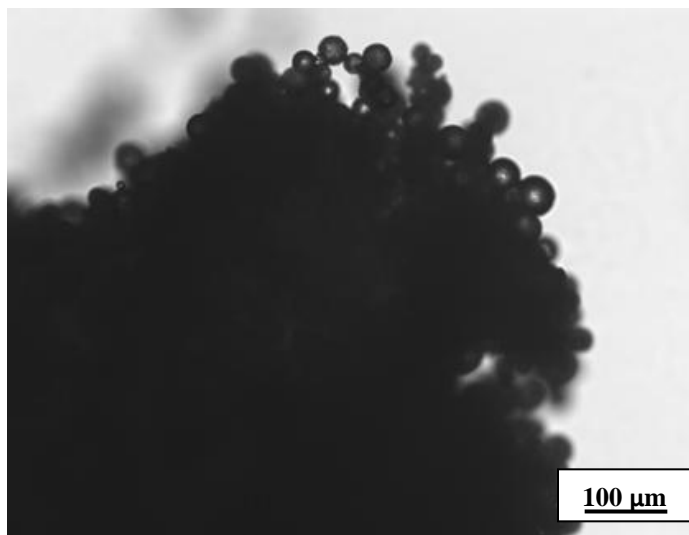


Figure 4.14: Large capsule aggregate (Scale bar is 100 μm)

In order to solve the re-dispersion problem, the industrial sponsor suggested that the use of a pilot spray dryer may be of benefit. Spray dryers are used very commonly in industry to prepare dry powders from dispersions [55, 56]. With our capsules, the purpose of the spray dryer would be to simply dry and separate the capsules before the aggregates have the opportunity to form. Figure 4.15 shows a spray dryer schematic and the relevant constituent components.

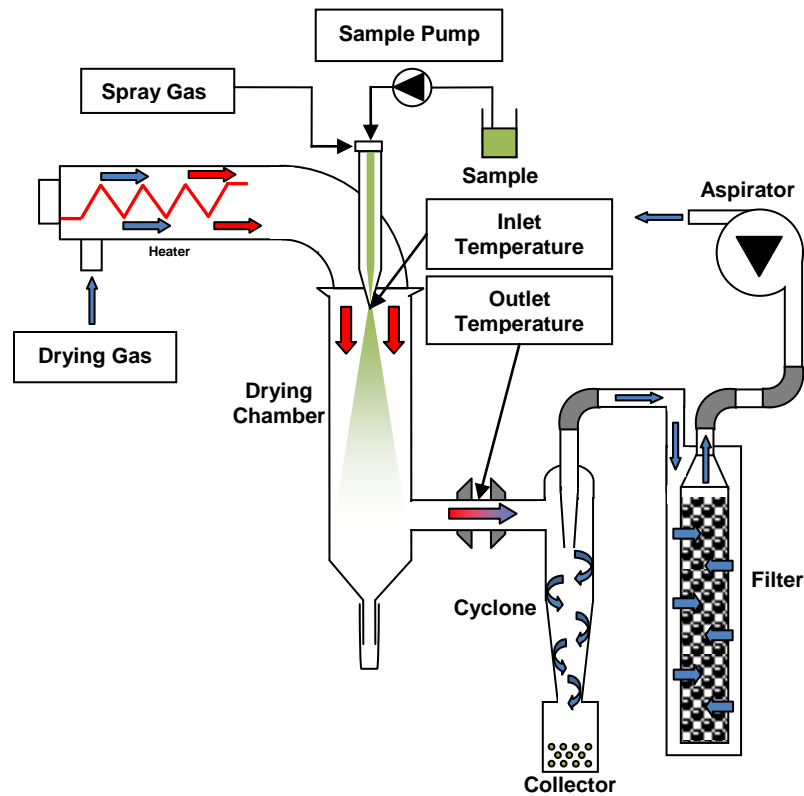


Figure 4.15: Schematic of a lab scale spray dryer

In 2014 a pilot spray dryer was provided by the industrial sponsor and sent to our lab, to address the capsule re-dispersion problem. Spray-drying conditions recommended by the industrial sponsor, which were developed for other encapsulation applications, were found to be unsuitable for our newly developed capsules. Setting the spray dryer's inlet temperature to 190-200°C proved too high. The capsules produced with the provided

spray drying conditions were dry, but they fused with one another and led to an unacceptably large effective capsule size. The fused capsules are depicted in Figure 4.16. We found that by reducing the inlet temperature to 150°C, which produced an outlet temperature of 90°C, and using the lowest possible feed flow rates, capsule fusion during spray drying could be prevented. The use of a spray dryer to dry the capsule dispersions proved adequate in ensuring that the capsules did not aggregate and that their resulting size was within the desired size range.

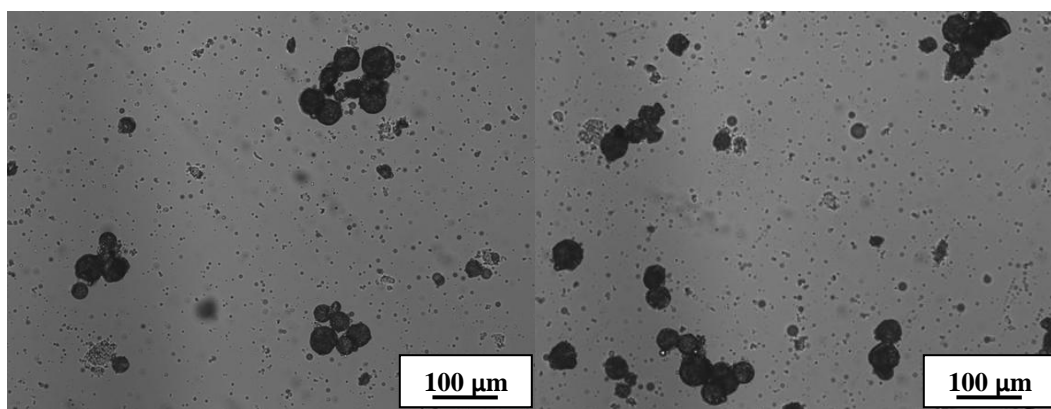


Figure 4.16: Failed re-dispersion of capsules that are fused due to high inlet temperatures during spray drying (Scale bar is 100 μm)

4.5.3.2 Minimum Capsule Loading of 10 wt%

It is important for the prepared capsules to contain a reasonable amount of the active ingredient. The loading criterion of 10 wt% was a preliminary value that would, at the least, show that the developed technique can produce capsules that have a reasonable amount of active. In reality, a loading greater than 10 wt% would be necessary for actual practical use of the capsules to be feasible. Loading of the capsules was determined in our lab by comparing the amount of active ingredient loaded into the emulsion mixture with the amount released when the capsules were washed after drying. At the time of the first

application tests of our capsules in the field, our measurements of the capsule loading values were around 10%. However, the initial test results from our industrial collaborators showed values that were much lower. Figure 4.17 shows the loading values from as measured by the industrial sponsor for samples of capsules that were prepared with different oil phases.




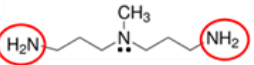
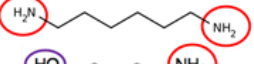
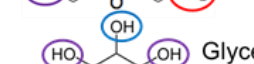
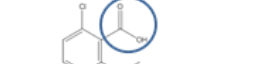

W/O/W Polyurea, Polyurethane capsules with TMPTMA, ca. 250mg Dicamba FD 130731-0029	W/O/W Polyurea, Polyurethane Capsules with Mineral Oil ca. 250mg Dicamba FD 130731-0030	W/O/W Polyurea, Polyurethane Capsules with Canola Oil ca. 250mg Dicamba FD 130731-0031
		
Dicamba Loading HPLC Results 3.8%	Dicamba Loading HPLC Results 4.5%	Dicamba Loading HPLC Results 4.6%

Figure 4.17: Loading results of the first samples sent for analysis to the industrial sponsor

This discrepancy in loading values likely occurred for two reasons. First, these initial samples were not completely dry and residual water increased the capsule weight. More complete drying of the capsules, via spray drying, resolved this problem. Second, the active ingredient may not be released in its entirety over the course of the release study. Our loading assessment was based on the amount of active added to the capsules in total, not the amount released over the course of a release study. Some of the active may react and become incorporated in the polymer shell. This active is encapsulated but may not be available for release until the capsule fully degrades. The knowledge of reactions that could prevent dicamba from being released subsequently meant that special attention

had to be paid to the reaction conditions in the internal aqueous phase. The reactivity of isocyanates with a variety of functional groups is shown below in Table 4.4.

Table 4.4: Relative reactivity of isocyanates with compounds present in the internal aqueous phase [52]

Group	Relative Reactivity @ 25°C	
Primary Aliphatic Amine	100,000	 BAPMA
Secondary Aliphatic Amine	50,000-20,000	 HMDA
Primary Hydroxyl	100	 DGA
Water	100	
Carboxylic Acid	40	 Glycerol & Water
Secondary Hydroxyl	30	 Dicamba

While the reactivity of dicamba with the isocyanate participating in the interfacial polyurea formation is small relative to the other possible reaction partners, the concentration of dicamba in the capsules is typically much higher than that of the polyamine (HMDA) to ensure a high active loading. This means that dicamba may successfully compete with the added amines and react with the isocyanate (PMDI) in the oil phase. Introducing the highly reactive N,N-Bis(3-aminopropyl)methylamine (BAPMA) as the cation in the dicamba salt (ENGENIA™ formulation) improved the capsule loading and adding excess BAPMA for the interfacial reaction improved the capsule loading even further. Table 4.5 shows the different loading weight percents measured by our collaborators. The increased active loading in ENGENIA™ capsules can likely be explained by a combination of higher active concentration in the encapsulated aqueous formulation (480 g/L in CLARITY® vs. 600 g/L in ENGENIA™), a smaller reactive loss of dicamba and better barrier properties of polyurea shells formed by the cross-linking of BAPMA. A loading of 14.9 wt% was confirmed for ENGENIA™ capsules with added BAPMA for the interfacial polymerization. The loading value of

14.9 wt% demonstrates that the developed technique can load the capsules beyond 10 wt% and it may be possible to further optimize the system and increase the loading further.

Table 4.5: Improvement of capsule loading assayed by industrial sponsor

Product	Product Active Ingredient(s)	Concentration (% w/w) Assayed	Date of Analysis
Spray Dried Clarity Capsules with HMDA	Dicamba (acid equivalent)	7.0	October 27, 2014
Spray Dried Engenia Capsules without added amines	Dicamba (acid equivalent)	9.7	October 27, 2014
Spray Dried Engenia Capsules with added BAPMA	Dicamba (acid equivalent)	14.9	October 27, 2014

4.5.3.3 Stability from 0-40°C Over 2 Weeks

Before the developed capsules are used, they will be stored for periods of time at either a manufacturing facility or on location at the farm where they are required. Temperature conditions are likely to vary as well as the duration of time that the capsules will be kept in storage. These conditions will have an impact on the capsule performance. During storage, some of the active may be released and it is important to determine the extent of this release as well as any other impact storage has on long term release of the active. Tests were performed with CLARITY® capsules stored at 0 and 40°C over the course of two weeks. Subsequent release studies were performed at room temperature. Figure 4.18 shows the cumulative release of dicamba from these capsules during the two weeks of storage as well as over a week long release study.

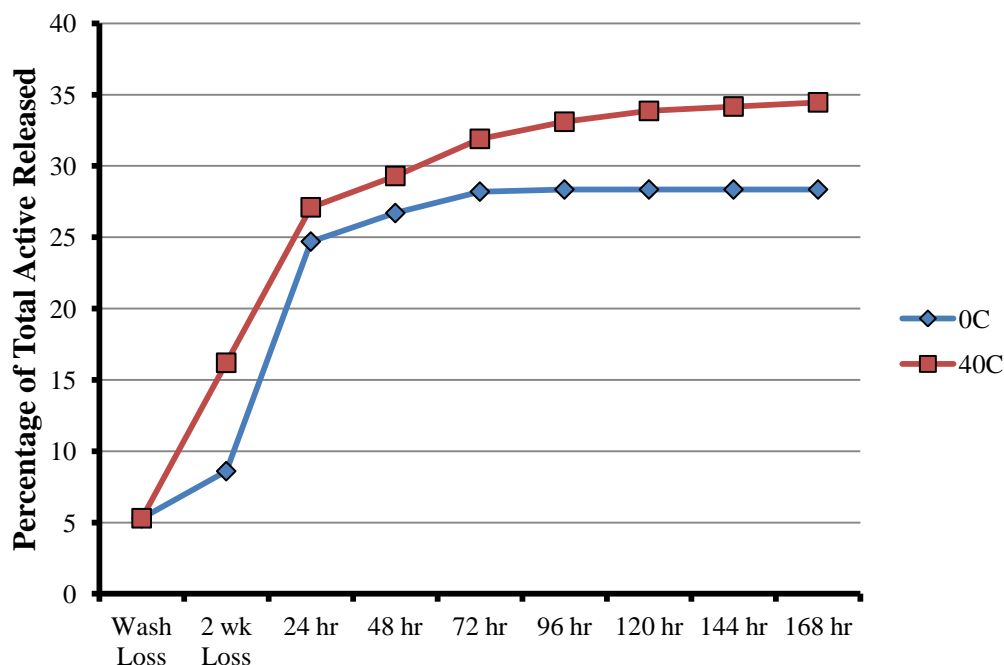


Figure 4.18: Release from CLARITY®/TMPTMA/water capsules upon and after storage at different temperatures.

During two weeks of storage, approximately 3.5% active is lost at 0°C and 11% is lost at 40°C. Over the course of the release study, performed at room temperature, the total amount of active released from the capsules is comparable. It is expected that there would be a greater amount of dicamba released during two weeks of storage at 40°C than at 0°C. The increased temperature could increase the diffusivity of dicamba and encourage a greater amount of release. The higher storage temperature of 40°C may degrade the polymer shell at a faster rate and account for a larger loss of dicamba during storage as well as a greater overall release of the active. The amount of active lost at 40°C can very likely be mitigated by using monomers that increase the degree of cross-linking at the interfacial polymer layers. Highly cross-linkable monomers would make degradation more difficult and also slow down the diffusion of dicamba from the capsule.

4.5.4 Release Performance

In the application area of agrochemicals, the use of microcapsules provides an opportunity to deliver a sustained amount of active ingredient. This reduces the frequency at which the active has to be applied and also reduces the amount of wasted active that doesn't reach the target. There were no set criteria in regards to expectations of release performance. But it was important to demonstrate that the developed capsules had some capacity for extended release and possibly the ability for the release profile to be tailored.

4.5.4.1 CLARITY® Capsules

CLARITY® was the first active formulation investigated for developing the encapsulation method. CLARITY® is a currently available commercial herbicide that has a dicamba-DGA salt as the active ingredient. The use of an active formulation like CLARITY® with the developed encapsulation method would demonstrate applicability to a currently used commercial product and hence the viability of the developed encapsulation method.

In order to sufficiently polymerize the capsule interfaces, stoichiometric amounts of monomer are required. A range of molar ratios of isocyanate to polyamine or polyol (from 1.2:1 to 0.92:1 respectively) were provided by the industrial sponsor as guidelines for interfacial polymerization conditions. Using these ratios at both the inner and outer interfaces, as well as some slight deviations, a series of capsules were made in order to probe if changing the molar ratio has a significant impact on the release profile. Figure 4.19 shows samples that were prepared within these ranges.

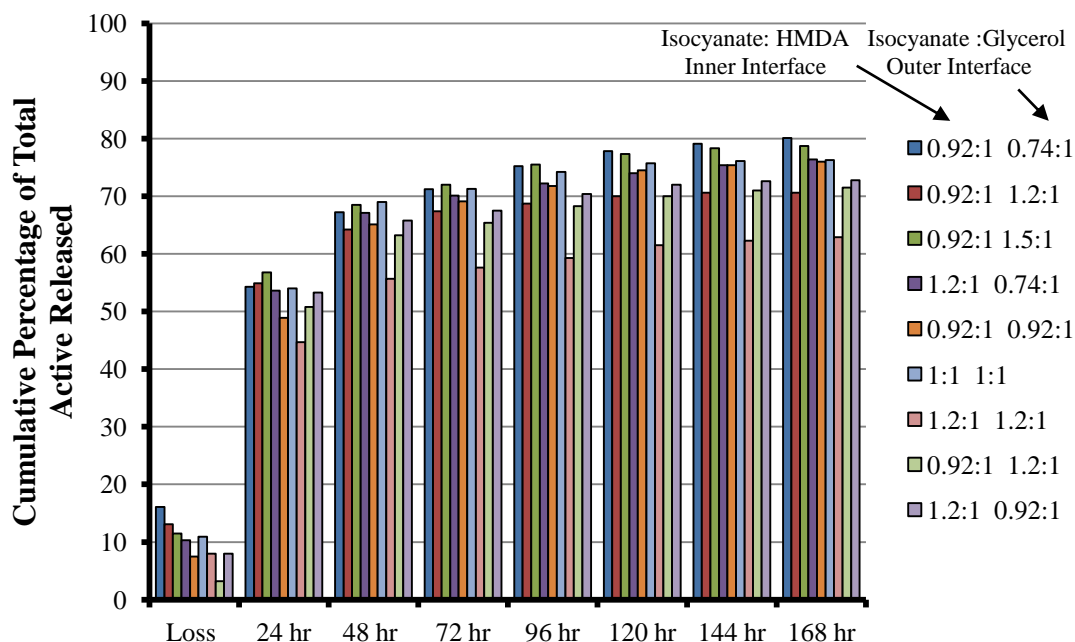


Figure 4.19: Release data of CLARITY®/TMPTMA/water capsules with varied molar ratios of monomers in suggested ranges and some deviations

Within the probed range of molar ratios, the release profiles do not appear to depend very sensitively on the reactant ratios. The total amount of active released in the monitored time ranges from 80% to 63% with the molar ratio of 0.92:1 & 0.74:1 leading to 80% release of the active while the molar ratio of 1.2:1 & 1.2:1 leads to 63% release. Increasing the isocyanate to HMDA ratio means that there will be an increased amount of side reactions with dicamba as well as DGA. This explains why the total amount of release differs between each of the samples. With this in mind, it seemed likely that adjusting the molar ratios further from the suggested ranges would lead to an increased variance in the total amount of active released. In the next set of experiments, the amount of isocyanate was reduced relative to the amounts of HMDA and glycerol in multiples of the 1:1 molar ratio. Figure 4.20 shows the release data of samples that were prepared with

increased amounts of glycerol for the outer interface and only increased amounts of HMDA for the inner interface.

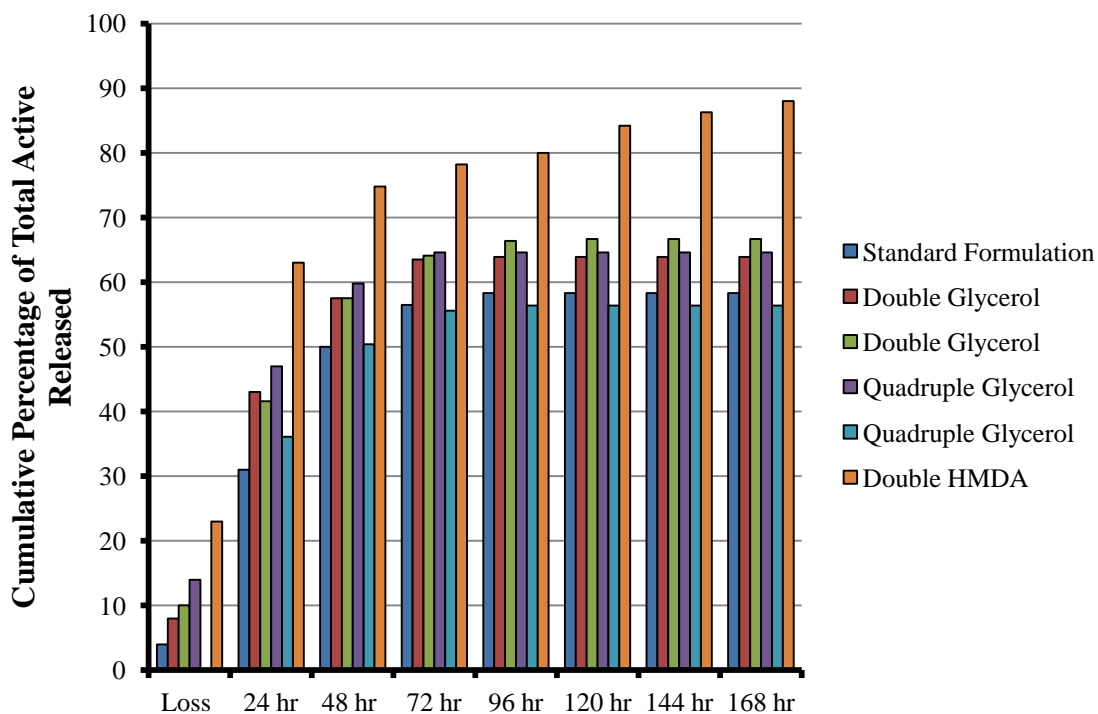


Figure 4.20: Release data of CLARITY®/TMPTMA/water capsules with varied amounts of glycerol or HMDA in the formulation

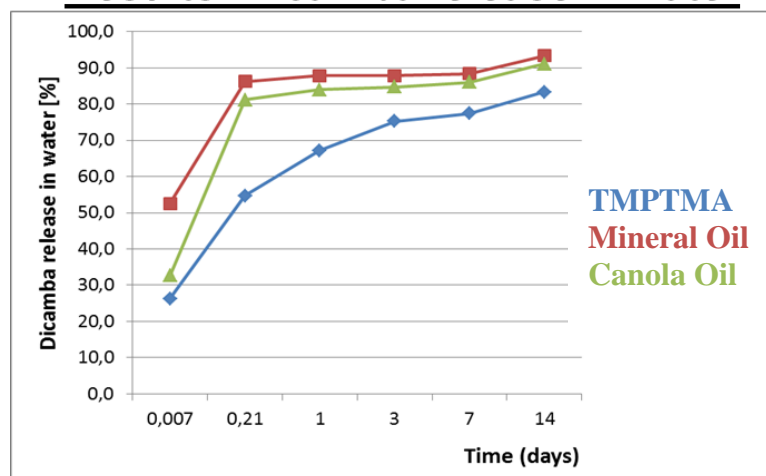
In these experiments, the total capsule release ranges from 56% to 88%. The greatest amount of release occurs when an increased amount of HMDA is added to the internal aqueous phase. An increased concentration of either monomer would intuitively lead to an increase in the thickness of the polymer layer up to the point that the diffusion limit is reached. However, when excess HMDA is used, the total amount of active released increases. This increase in release implies that an alternative reaction phenomenon is at play. The presence of excess HMDA likely reduces the amount of dicamba and DGA that can react with the isocyanate to a point that ensures that almost the entire amount of the active is released over the course of the release study. Additional

glycerol was added as a means to possibly slow the release of the active by making a thicker polyurethane layer on the outer capsule interface. The results show that the impact of an increased glycerol concentration is minimal. This indicates that the reaction is likely diffusion limited and that increased concentrations of glycerol have little impact on the polyurethane layer thickness.

Biological tests were performed on the first set of interfacially polymerized capsule samples. These are the same samples that were tested earlier by the industrial sponsor that had low active loading values (shown in Figure 4.17). The only difference between these samples was the oil phase that was used. Only three oil phase samples were tested; mineral oil, canola oil and TMPTMA. Mineral oil and canola oil were tested as more environmentally friendly oil phase alternatives to TMPTMA. Figure 4.21 shows results from both long term release tests and biological efficiency tests comparing the performance of these different capsules.

The release results show that TMPTMA capsules display the slowest release relative to the mineral and canola oil capsules. Consistent with this slower release, capsules prepared with TMPTMA showed a slightly higher biological efficacy than the other capsules in weeks 2-4. Within the first 3 weeks, none of the capsule formulations showed any benefit over the un-encapsulated CLARITY® solution. In week 4 however, the capsules prepared with TMPTMA retained about 15% biological efficiency, whereas the un-encapsulated solution retained no significant efficiency. This was a modest success, but nonetheless a promising result for the first generation of capsules tested.

Results: Dicamba release in water



Results: Biological efficiency over weeks

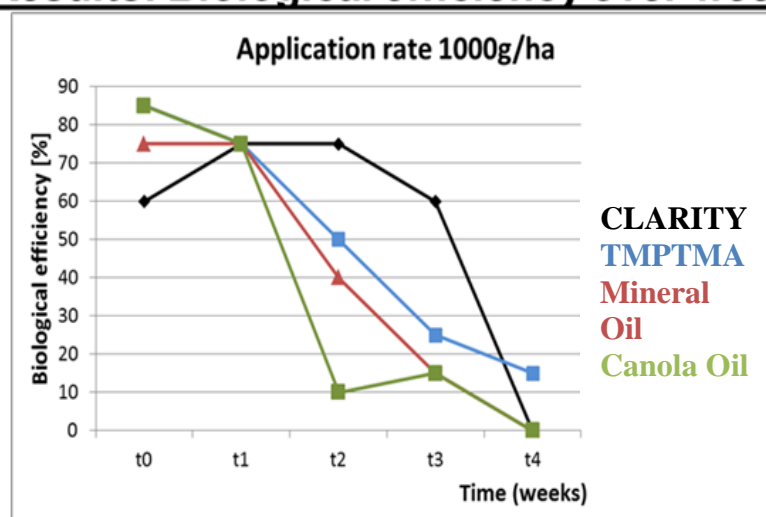


Figure 4.21: Release data (top) of CLARITY® capsules with different oil phases and biological efficiency data (bottom, measured by the industrial sponsor) of CLARITY® capsules containing different oil phases in comparison with un-encapsulated CLARITY®.

4.5.4.2 ENGENIA™ Capsules

The promising results from the first generation of CLARITY® capsules indicated that a slower release and biological efficiency are directly related. In an attempt to slow down the release of dicamba from the capsules further and hence improve the long-term

biological efficiency, an alternative dicamba formulation (ENGENIA™) was suggested. ENGENIA™ uses BAPMA instead of DGA as the counter-ion for dicamba. As mentioned before, BAPMA is more reactive and can serve as a cross-linker that would aid in forming a less permeable polyurea layer on the inner W/O interface. Similar to the CLARITY® capsules, the bulk of the release happens during the first 24 hours. Encapsulation efficiency is typically 90% or above due to the increased rate of polyurea formation. In addition, the use of ENGENIA™ in the capsule formulation significantly improves the capsule loading relative to CLARITY® (previously shown in Table 4.5). ENGENIA™ capsules can be prepared with no additional polyamine due to the cross-linking capabilities of BAPMA present in the formulation. But, as shown in Figure 4.21, only a little over 20% of the loaded dicamba is accounted for over the course of a typical release study with these capsules. These data alone do not explain whether the active that is unaccounted for remains bound to the capsule walls and would be released at a later time or whether it is already lost prior to re-dispersion of the dried capsules. It seems plausible that side reactions occur when BAPMA from the ENGENIA™ formulation participates in the interfacial polymerization and is thus no longer available as a counter-ion to the de-protonated dicamba. It would seem logical that adding more BAPMA would increase the total amount of active released. Unfortunately, only a small amount of excess BAPMA can be added without causing a severe thickening of the oil phase which increases the maximum capsule size above the 50 µm limit. An alternative approach would be to use a less reactive monomer that can still serve as a counter-ion to dicamba. HMDA, the polyamine monomer used in CLARITY® formulations, was explored as a possible alternative. ENGENIA™ capsules were prepared with added HMDA added

instead of excess BAPMA and compared to the aforementioned capsules that had no additional monomer in them. The results of the release tests are shown in Figure 4.22.

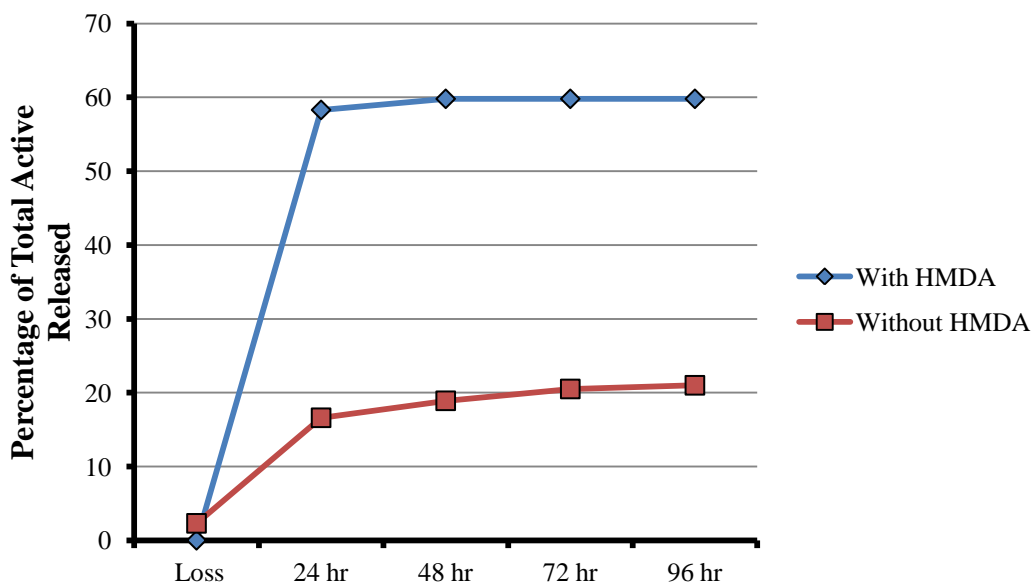


Figure 4.22: Release data of ENGENIA™/TMPTMA/water capsules with and without HMDA

The results show that the use of HMDA as an alternative monomer in the ENGENIA™ capsule formulation increases the total amount of active released from the capsule to about 60%. The release profile of the ENGENIA™ capsules containing HMDA is not a profile that would be beneficial for biological efficiency. While most of the active is released during the first 24 hours, which is similar to all capsule formulations, there is little to no release subsequently after. The CLARITY® capsules that had the best biological efficiency had a release profile that steadily released the active over the course of the study. The observed sensitivity of the release profile to the type of amine monomer used for the interfacial polymerization is nonetheless interesting and warrants further investigation.

4.5.4.3 Impact of Excess Water

HMDA is a very corrosive polyamine. For this reason it was suggested by the industrial sponsor that solutions of HMDA be diluted to 25 wt% with water. When capsule samples were prepared that required more HMDA, an increased volume of the HMDA solution was used. This created problems due to the higher concentration of water present in the formulation. When the concentration of water is too high (greater than 25 vol% HMDA solution in the internal aqueous phase), a significant amount of water can react with the isocyanate and produce CO₂ gas. The resulting volume expansion can rupture the capsules or lead to the formation of “tails” as seen in Figure 4.23. Instead of increasing the total volume of the HMDA solution, HMDA was diluted less and even used in its highly concentrated form (70% HMDA) to minimize side reactions of isocyanate with water.

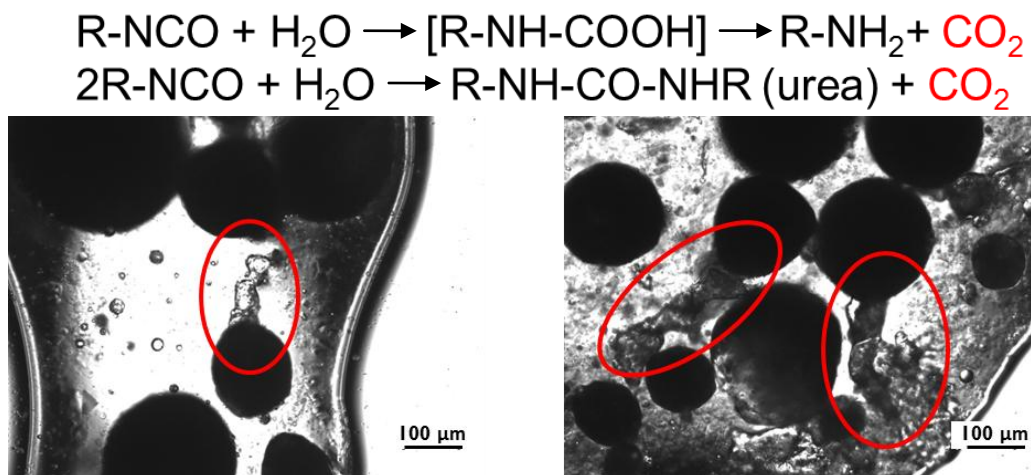


Figure 4.23: Chemical reactions (top) that can lead to the capsule rupture or the formation of “tails” (bottom left and right)

Cautionary measures were taken to ensure that contact with concentrated HMDA was minimal and the issues associated with a higher concentration of water were avoided.

Volume percents of the HMDA solution that were around 25 vol% also caused buckling of the internal W/O shell. Figure 4.24 shows how the reduction in the vol% of HMDA solution changes the resulting shape of the W/O shell. It is plausible that due to the dilution of the HMDA solution, the diffusion limit of the polyurea interface has not been reached. As a result, HMDA is still able to diffuse out of the shell and cause it to contract and reduce in volume. In order to maintain the round inner shell shape and minimize the formation of “tails”, the vol% of HMDA solution was kept around 10 vol%.

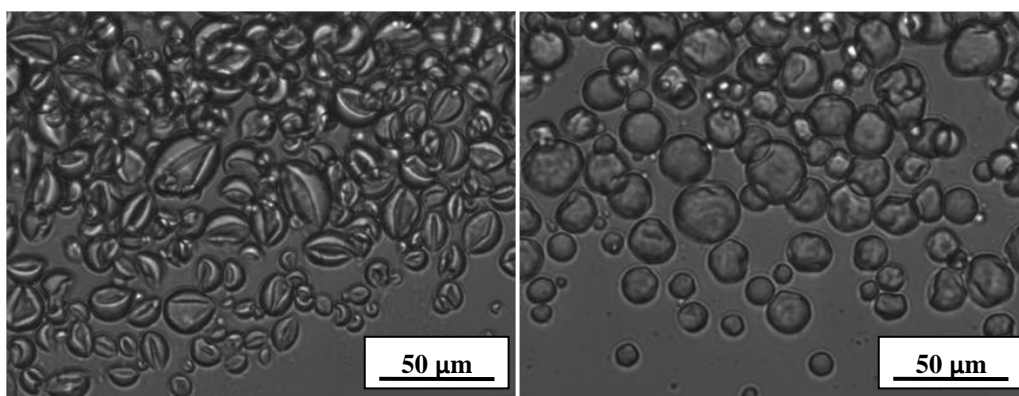


Figure 4.24: Inner W/O polyurea shells with 25 vol% HMDA solution (left) and 10 vol% HMDA solution (right) (Scale bar is 50 μm)

4.5.4.4 Counter-ion Type and Volatility

Dicamba formulations contain counter-ions that play an important role in reducing the volatility of dicamba and improving its storage stability. In CLARITY®, the counter-ion is DGA and in ENGENIA™, the counter-ion is BAPMA. Even though the vapor pressure of dicamba in its free form is 2×10^{-5} mmHg at 25°C, substantial injury to non-targeted plants can occur when it is applied to the field [57]. In order to reduce this unwanted volatility effect, it is essential that the counter-ion to dicamba remains in the formulation in the highest possible concentration. According to the reactivity data (shown

in Table 4.4), side reactions that consume the counter-ions can occur when isocyanates are used in the oil phase to create an interfacial polymer layer. This means that there is risk of increased dicamba loss due to increased volatility; which would defeat the entire strategy of encapsulating the active. If an excessive amount of the active is volatilized then it will not be available for subsequent release to the target. This is an alternative explanation to the incomplete release of the active that occurs when less polyamine is used in the internal aqueous phase. The importance of side reactions with the counter-ion became apparent in May 2014 when the results of volatilization experiments for the capsule samples performed by the industrial sponsor showed increased volatility of encapsulated dicamba relative to the un-encapsulated dicamba salts. These results are shown in Table 4.6.

Table 4.6: Results of dicamba volatilization experiments performed over 24 hours

Sample ID	Dicamba Wt% Loss
Dicamba DGA salt	1.9
Dicamba BAPMA salt	0.4
Capsules of dicamba DGA (CLARITY) + HMDA	54.5
Capsules of dicamba BAPMA (ENGENIA)	12.0

Figure 4.25 shows SEM images of the capsules which further revealed that most of the capsules containing dicamba-DGA (CLARITY®) and added HMDA were broken, whereas many capsules containing only dicamba BAPMA (ENGENIA™) without added monomer were still intact. In the absence of any additional monomer, ENGENIA™ capsules still manage to have less volatilization of dicamba and produce capsules that maintain their shape.

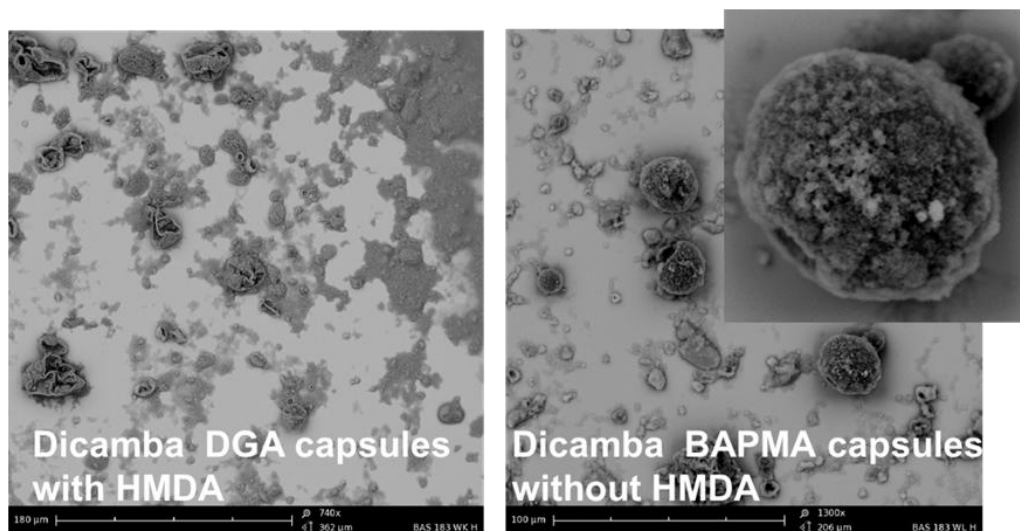


Figure 4.25: SEM images of CLARITY® and ENGENIA™ capsules. Most CLARITY® capsules (left) are broken, hypothetically due to an ion exchange between DGA and HMDA. By contrast many ENGENIA™ capsules (right) remain intact and spherical, with a typical capsule size around 25 µm.

This observation suggests that, in CLARITY® capsules, side reactions with the counter-ion DGA not only increase dicamba volatilization but weaken the integrity of the capsule overall. DGA possesses a highly reactive amine group and a less reactive alcohol group. Rapid reactions of the isocyanate with DGA, due to the amine group, limit the total amount of polyurea that can form. This means that the inner W/O interface does not get an opportunity to polymerize fully, and will have less structural integrity.

Based on these observed differences, we expected that the ENGENIA™ capsules could be further improved with regard to active ingredient volatility and capsule stability. This could be done by adding more BAPMA to the formulation so that the BAPMA counter-ions are not consumed by the polymerization reaction. ENGENIA™ capsules were thus prepared with added BAPMA in the aqueous core phase at a mole ratio of 0.22:1 of BAPMA to diisocyanate (LUPRANATE® M20) in the oil phase. This mole ratio is the maximum amount compatible with a maximum capsule size of 50 µm and that

can be rendered re-dispersible by spray drying. These capsules were found to have the highest active ingredient loading of all tested samples (14.9 wt%, shown in Table 4.5) and were expected to be the best candidates for sustained release and biological efficiency tests.

4.5.4.5 Final Application Tests

Nonetheless, biological performances tests on the ENGENIA™ capsules with added BAPMA, the last of which were completed in April 2015, showed disappointing results. Figure 4.26 shows the results of these tests on different plant species.

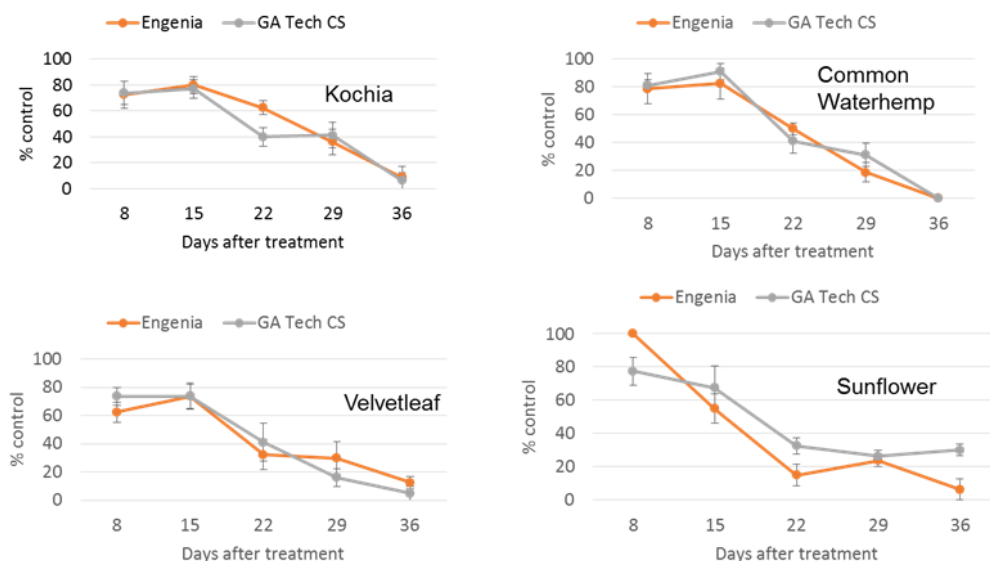


Figure 4.26: Biological Persistence Assay of dicamba formulations applied at 1120 g ae/ha for different plant species.

Within four weeks of treatment by either ENGENIA™ or the encapsulated formulation, residual weed control experiments on different plant species showed essentially no difference between the encapsulated and un-encapsulated formulations. The only slightly positive result came from tests done sunflower plants. Since these latest capsules formulations were produced at the very end of the project period and the final

test results were obtained after project completion, it has not been possible to fully investigate the reasons for the perceived capsule failure. Although it cannot be excluded that spray-drying the capsules may have compromised the capsule integrity, it seems more likely that inherent shortcomings of the formulation protocol are to blame.

4.5.4.6 The Achilles' Heel of the Chosen Encapsulation Approach

As for the potential source and nature of any “inherent weakness” in our encapsulation strategy for aqueous dicamba formulations, our study points to an obvious suspect: the interaction of the dicamba or its counter-ion with the reactants forming the inner polyurea capsule shell. The choice of interfacial polyurea formation as a way to protect the aqueous cargo clearly has many advantages. However, an interfacial reaction involving amines may not be the best choice when the cargo to be protected and preserved contains itself highly reactive amines such as BAPMA. In hindsight, the developed encapsulation method may have been better suited for encapsulating active ingredients that did not contain amine salts. Many of the biggest challenges encountered in this project can be traced back to the interference of the dicamba salt with the interfacial polymerization. It also seems plausible that the release properties and application performance of the capsules are also affected. If this is true, the encapsulation strategy developed in this project may yet prove very useful for the formulation of active ingredients that do not contain reactive amines.

4.6 Conclusions

We have demonstrated that particle-stabilized double emulsion droplets can be useful templates for capsules with an aqueous core dispersed in an aqueous medium. We found that reversible osmotic swelling affords some control of the droplet morphology in

multiple emulsions produced by batch emulsification. In particular, it allows for the production of double W/O/W emulsion droplets with a single aqueous core that can otherwise be achieved only in continuous emulsification processes. Polymer capsules templated by the particle-stabilized double emulsion droplets were formed by bulk polymerization of the oil phase or by interfacial polymerization. Polyurea formation at the inner W/O interface and polyurethane formation at the outer O/W interface were carried out to produce capsules with two particle-polymer composite shells. A systematic comparison with similar capsules template by surfactant-stabilized double emulsions demonstrated a clear benefit of the particles embedded in the capsule shell. Two patent applications have been filed for the emulsion morphology control by osmotic swelling and for the water-in-water encapsulation by double interfacial polymerization of Pickering double emulsions. Capsule formulations in the targeted size range of 1 - 50 μm have been achieved, and a characteristic trade-off has been identified between capsule size reduction and the maximization of capsule loading and active ingredient retention via incorporation of cross-linking BAPMA in the inner polymer shell. The highest active loading achieved for capsules with a maximum size of 50 μm was 14.9% which exceeded the target of 10% overall loading. It was also shown that temperatures of 0⁰C or 40⁰C did not dramatically compromise the formulation stability during 2 week storage. The active ingredient release from capsules into pure water was studied for different capsule compositions, and initial formulations of non-spray-dried capsules containing dicamba DGA showed sustained biological activity after four weeks in field tests. Capsule formulations were found to suffer, however, from enhanced dicamba volatility and poor capsule stability caused in all likelihood by side reactions between the diisocyanate and

the counter-ion of the dicamba salt. Capsules of dicamba-BAPMA (ENGENIA™) formulated with excess BAPMA to address these problems and spray-dried for good re-dispersibility surprisingly did not fare better in application tests than the un-encapsulated formulation. While this particular failure may be the result of damage to the capsules during the spray drying process, it must be concluded that formulations of dicamba-amine salts with the explored type of encapsulation are very sensitive to interactions with the reactants for the interfacial polyurea formation. Better results may be expected for amine-free active ingredients.

CHAPTER 5

FUTURE RESEARCH DIRECTIONS

5.1 Membrane Emulsification

As was mentioned earlier in the thesis, there are methods that can easily produce monodisperse double emulsion droplets. The problem with industrial utilization of these methods is that they require new equipment to make the product and they do not have sufficiently high throughputs. Membrane emulsification is a technique that only suffers from the former problem but has been shown to enable the production of industrially viable quantities of double emulsions [58-60]. Membrane emulsification is a continuous process that generates droplets from the pores of the membrane as one of the emulsion phases passes through. The droplets that are produced from the membrane are then dispersed in the surrounding bulk medium to make an emulsion provided there is an emulsion stabilizer present. The resulting droplets are not exactly monodisperse, but there is an intermediate level of control over the droplet size which is much better than the homogenization methods we used. The homogenization methods that we used to produce emulsions in bulk can only produce small droplet sizes, and by extension, small capsule sizes, if sufficiently strong shear forces are used to shear existing droplets. The strong shear forces that are required to produce small double emulsion droplets can excessively shear the double emulsion during the second emulsification step. Continuous membrane processes that directly generate the droplets in their final size could avoid this problem and guarantee a fairly consistent droplet size.

It is highly feasible, based on the work already done in membrane emulsification, that the methodology used to make capsules described in Chapter 4 can be generated with

membranes. Initial experiments could use SPG (Shirasu Porous Glass) membranes which have been used at the lab scale to produce double emulsions that were used as templates for capsules [61-63]. Producing capsules in the desired size range of 1 – 50 μm was one of the biggest challenges of the industrially sponsored project. The alternative approach of membrane emulsification should be pursued if other encapsulation projects involving double emulsions are pursued.

5.2 Particle Modification

While silanization is a common method of surface modification, it is not a method that our lab has extensive experience with. Surface modification in our lab is typically done for the specific purpose of modifying the hydrophobicity of particles to improve the stability of a particular emulsion or foam. In the project described in Chapter 3, surface modification served the purpose of both increasing the hydrophobicity of silica particles and preferentially adsorbing the particles to specific liquid interfaces. The development of particles that have significant preferential adsorption to specific liquid interfaces, and hence specific changes to interfacial tension in a multiphase system, is an area where an opportunity exists to create a new research project.

We were very lucky that the surface modifications that we used were capable of demonstrating a significant change in droplet morphology. If it is desirable to create a robust method of changing droplet morphology, much more work has to be done. There are many different silanes that vary in their molecular weight and the functional groups they contain. Each liquid phase that is used will differ in its solubility for each respective silane which in turn changes the adsorption preference of the particle modified with the silane. The complexity is increased when you involve three liquid phases since at least

two of the phases are oils that are slightly soluble in each other and the silane is likely to also be soluble in more than one phase. It is innately challenging to tailor specificity of particles using this methodology.

It is still unclear how using a single silane as a surface modifier changes the particle contact angle at different liquid interfaces. Goedel et al. have performed experiments with silica particles modified with four different silanes [28]. But the extent of surface modification was not reported and it can drastically change the wettability of a particle modified with just one silane [43]. Quantifying the extent of surface modification requires a methodology to quantify the silanol concentration on the particle surface which is luckily a well-established technique. [64]. In addition, Goedel et al. used glass surfaces modified with the silanes to quantify the contact angles of silica surfaces modified with the same silanes. While this is an easy way to acquire contact angle results, the results may not correlate well with the actual contact angles of the silica particles. Gel-trapping methods are much more accurate but are much more involved [46, 47]. Gel trapping methods also require larger particles to be prepared since they need to be optically visible via SEM. While there may be large silica particles available commercially, it is possible to synthesize monodisperse silica particles “in house” using the Stober process [65-67]. A systematic study of particle modification using different silanes, quantifying the extent of surface modification, quantifying the particle contact angles at the liquid interfaces and then measuring the impact on the interfacial tension should begin to demonstrate what the limitations are in regard to modifying particles for the purpose of preferential interfacial adsorption.

5.3 Tailoring Capsule Release

During the industrially sponsored project, we successfully developed a novel method by which aqueous agrochemical formulations could be encapsulated and used to gradually release the active to the target. Unfortunately, we only discovered one method by which the release profile of the active ingredient could be changed. Changes in the release profile of the active ingredient were achieved by the addition of more or less of the monomer used in the internal aqueous phase. Changing the amount of monomer in the internal aqueous phase only increased or decreased the total amount of active released but did not change the rate at which the release occurred. The effect of changing the monomer, in our case from HMDA to BAPMA, was masked by an inability to produce capsules of a sufficient size due to a rapid polymerization that made the emulsion too thick to shear. Despite the aforementioned hurdle, there is still justification to investigate the use of other monomers of differing molecular weights and cross-linkability. Initial studies could start by performing FRAP (Fluorescence Recovery After Photo-bleaching) studies on single polyurea capsules prepared with different monomers. FRAP studies have been performed in our group previously to quantify the permeability of individual microcapsules [68]. If the capsule permeability can be changed significantly with different monomers, then there is an additional method by which the release of the active ingredient from the capsule can be controlled.

5.4 Lutidine-Water Encapsulation

We came across a recent high profile publication that used the two phase system of lutidine and water to investigate critical Casimir forces [69]. We currently have no interest in measuring Casimir forces. However, the lutidine-water system is an appealing

system from an emulsion and possible encapsulation perspective. The lutidine-water system is a unique two phase system that is miscible at room temperature and has a relatively low LCST of 34°C. As the LCST is approached at low lutidine concentrations, small droplets of lutidine begin to form. Once the phase boundary has been crossed, either a bijel (short for bicontinuous, interfacially jammed emulsion gel) or an emulsion can form depending on the weight percent of lutidine in the mixture. Cooling the mixture back down to room temperature returns the mixture to its initial mutually miscible state. Emulsions of lutidine-in-water or vice versa can be prepared with very small droplets by simply heating a mixture of lutidine-water that has either a high concentration of water or lutidine respectively. Adding particles to the lutidine-water mixture would then make it possible to stabilize the emulsion droplets which, from preliminary studies, remain even after the mixture is cooled to room temperature. In order for emulsions to be prepared at high enough yields however, more detailed phase data is required. There are very clear phase diagrams for the lutidine-water system even in the presence of silica particles that describe behavior below the coexistence curve [70]. The lack of data in the region of immiscibility is of interest and has only been depicted [71]. The region above the coexistence curve should be mapped to clearly separate the emulsion regions for the bijel region. Additional studies can be performed using oil phases that differ in their miscibility for lutidine and water. For example, an oil phase that is immiscible in water but miscible in lutidine at elevated temperatures will ultimately remain encapsulated when the mixture returns to room temperature. The logistics and impacts on the phase diagram are unknown for an experiment of this nature. However, if this topic is pursued,

very small emulsion droplets can be produced without the use of shear from a homogenizer.

Attempts to observe interfacial phenomenon in this system in the presence of particles were attempted using a Nanosight NTA instrument in our lab. The Nanosight instrument is a particle tracking analyzer that records videos of particles in solution and then calculates their size based on their displacements when they are applied to the Stokes-Einstein equation. Unfortunately, the device has no temperature control. If the technical challenge of temperature control can be overcome, an opportunity exists to quantify changes in the apparent particle size due to changes in the solubility of the phases. In addition, video can be captured that would display the behavior of these particles as they approach interfaces that appear and disappear due to temperature changes. The aforementioned phenomenon has never been observed or characterized in with a particle tracking analyzer. The results from such as study would add new fundamental understanding to the lutidine-water system in the presence of particles.

APPENDIX A

PHASE DIAGRAM OF MACROSCOPIC SPREADING WITH PARTICLES

To generate the phase diagram for a macroscopic three phase system, the five equations that represent each of the possible morphologies have to be solved. The equation that produces the lowest value for the system free energy at a given set of contact angles is the equilibrium morphology. The equations for the five possible morphologies are expressed below.

Complete Incorporation into lenses:

$$\frac{\Delta E}{\pi r^2 \gamma_{aw}} = -4 \frac{\gamma_{ao}}{\gamma_{aw}} \cos \theta_{aop}$$

Dry Particle Monolayer and pure oil lenses:

$$\frac{\Delta E}{\pi r^2 \gamma_{aw}} = -(1 + \cos \theta_{awp})^2$$

Wetting layer, particles at the top interface:

$$\frac{\Delta E}{\pi r^2 \gamma_{aw}} = -\frac{As}{\pi r^2 \gamma_{aw}} - \frac{\gamma_{ao}}{\gamma_{aw}} (1 + \cos \theta_{aop})^2$$

Wetting layer, particles at the bottom interface:

$$\frac{\Delta E}{\pi r^2 \gamma_{aw}} = -4 \cos \theta_{awp} - \frac{As}{\pi r^2 \gamma_{aw}} - \frac{\gamma_{wo}}{\gamma_{aw}} (1 + \cos \theta_{wop})^2$$

Wetting layer, particles at the both interfaces:

$$\frac{\Delta E}{\pi r^2 \gamma_{aw}} = -2 \cos \theta_{awp} - \frac{As}{2 \pi r^2 \gamma_{aw}} - \frac{\gamma_{wo}}{2 \gamma_{aw}} (1 + \cos \theta_{wop})^2 - \frac{\gamma_{ao}}{2 \gamma_{aw}} (1 + \cos \theta_{aop})^2$$

The additional expression, $\gamma_{aw}\cos\theta_{awp} - \gamma_{ao}\cos\theta_{aop} + \gamma_{wo}\cos\theta_{wop} = 0$, makes it possible to reduce the number of independent variables to two. The air phase can be treated as the bulk phase, the water phase can be treated as the engulfed phase and the oil phase can be treated as the spreading phase.

The m-file below will generate a phase diagram using the five possible morphology equations. To use the m-file, simply copy and paste the m-file into the MATLAB m-file editor and press F5 or click run to start the calculation and generate the phase diagram.

```
function Coexistence3
%Generate phase diagram of spreading behavior%
%With color%
clc
clear all
close all
%Engulfed Phase is Phase 1%
%Bulk Phase is Phase 2%
%Spreading Phase is Phase 3%
gao_gwo=50/8; %(interfacial tension of 2-3 interface)/(interfacial
tension of 1-3 interface)%
So_gaw=-5/55; %(spreading coefficient of spreading phase)/(interfacial
tension of 1-2 interface)%
phi=0.91; %particle packing fraction (maximum of 0.91%
V=(pi/phi)^2;
A_r2=sqrt(V);
gao_gaw=gao_gwo*((1-So_gaw)/(gao_gwo+1));
gwo_gaw=(1-So_gaw)/(gao_gwo+1);
N=200;
thetaaop=linspace(0,pi,N);
thetawop=linspace(0,pi,N);
indx=zeros(N,N);
hold on;
for i=1:N
    for j=1:N
        %Complete Incorporation%
        mat(1)=-4*gao_gaw*cos(thetaaop(j));
        %Dry Monolayer%
        mat(2)=-(1+gao_gaw*cos(thetaaop(j)))-
gwo_gaw*cos(thetawop(i))^2;
        %Wetting Layer Particles on Top%
        mat(3)=-gao_gaw*(1+cos(thetaaop(j)))^2-A_r2*So_gaw/pi;
        %Wetting Layer Particles on Bottom%
        mat(4)=-gwo_gaw*(1+cos(thetawop(i)))^2-A_r2*So_gaw/pi-
4*(gao_gaw*cos(thetaaop(j))-gwo_gaw*cos(thetawop(i)));
```

```

        %Wetting Layer Particles on Top & Bottom%
        mat(5)=-gao_gaw/2*(1+cos(thetaaop(j)))^2-
        gwo_gaw/2*(1+cos(thetawop(i)))^2-A_r2*So_gaw/(2*pi)-
        2*(gao_gaw*cos(thetaaop(j))-gwo_gaw*cos(thetawop(i)));
        [c,indx(i,j)]=min(mat);
        if indx(i,j) == 1
            plot(thetawop(i).*180./pi,thetaaop(j).*180./pi,'r.')
        elseif indx(i,j) == 3
            plot(thetawop(i).*180./pi,thetaaop(j).*180./pi,'g.')
        elseif indx(i,j) == 4
            plot(thetawop(i).*180./pi,thetaaop(j).*180./pi,'y.')
        elseif indx(i,j) == 5
            plot(thetawop(i).*180./pi,thetaaop(j).*180./pi,'b.')
        end
    end
end
title(['\gamma_2_3/\gamma_1_3 = ' num2str(gao_gwo) ' ' S_3/\gamma_1_2
= ' num2str(So_gaw) ' '\phi = ' num2str(pi/sqrt(V))'],'FontSize',18)
xlabel('\theta_1_3_p','FontSize',18)
ylabel('\theta_2_3_p','FontSize',18)
axis square
axis([0 180 0 180])

```

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